

1 agggagaggc agtgaccatg aaggctgtgc tgcctgccc tngatggca
 51 ggctggccc tgcagccagg cactgcccctg ctgtgctact cctgcaaagc
 101 ccaggtgagc aacgaggact gctgaggtt ggagaactgc acccagctgg
 151 gggagcagtg ctggaccgctg cgcattccgctg cagtggcct cctgaccgtc
 201 atcagcaaag gctgagctt gaactgcgtg gatgactcac aggactacta
 251 cgtgggcaag aagaacatca cgtgctgtga caccgacttg tgcaacgcc
 301 gcggggccca tgcctgagc cgggtgccc ccatcctgc gctgctccc
 351 gcactcggcc tgcctgctg gggaccggc cagctatagg ctctgggggg
 401 ccccgctgca gccacactg ggtgtgtgtc cccaggcctt tgtgccactc
 451 ctacagaac ctggcccagt gggagcctgt cctggctcct gaggcacatc
 501 ctaacgcaag ttgaccatg tatgtttgca cccctttcc cnaaccctg
 551 accttccat gggcctttc caggattcct accnaggcaga tcagtttag
 601 tganacana cgcctgagc atggccctc caacncttt tgttgnrt
 651 tccatggccc agcatttcc accttaacc ctgtgtcag gcactttc
 701 cccaggaag cctccctgc ccacccat tatgaattga gccaggttg
 751 gtccgtgtgt tccccgcac ccagcagggg acaggcactc aggagggccc
 801 agt222ggct gagatg22gt ggactgagta g22ctggagg ac22ag22g
 851 acgtgagctc ctgggagctt ccag22at22 g22ctggagg cctgg22g22
 901 ggggcccaggc ctacattg tggggctccc g22tggcagc ctgagc22g
 951 cgtaggccct t22t22cac ctgnggata agcc22222 2222222

FIGURE 1A

MRALLALLMAGLALQPGTALLCYSCKAQVSNEDECLQV
ENCTQLGEQCWTARIRAVGLLTVISKGCSLNCVDDS
QDYVVGKKNITCCDLDLCNASGAHALQPAAAILALLPAL
GLLLWGPGL

FIGURE 1B

1 ATGAAGACAGTTTTTTTTATCCTGCTGGCCACCTACTTAGCCCTGCATCCAGGTGCTGCT
 TACTTCTGTCAAAAAAATAGGACGACCGGTGGATGAATCGGGACGTAGGTCCACGACGA 60
 M K T V F F I L L A T Y L A L H P G A A
 61 CTGCAGTGCTATTTCATGCACAGCACAGATGAACAACAGAGACTGTCTGAATGTACAGAAC
 GACGTACAGATAAGTACGTGTCGTGTCTACTTGTGTCTCTGACAGACTTACATGTCTTG 120
 L Q C Y S C T A Q M N N R D C L N V Q N
 121 TGCAGCCTGGACCAGCACAGTTGCTTTACATCGCGCATCCGGGCCATTGGACTCGTGACA
 ACGTCGGACCTGGTCGTGTCAACGAAATGTAGCGCGTAGGCCCGGTAACCTGAGCACTGT 180
 C S L D Q H S C F T S R I R A I G L V T
 181 GTTATCAGTAAGGGCTGCAGCTCACAGTGTGAGGATGACTCGGAGAACTACTATTGCGG
 CAATAGTCATTCCCGACGTCGAGTGTCACTCTACTGAGCCTCTTGATGATAAACCCG 240
 V I S K G C S S Q C E D D S E N Y Y L G
 241 AAGAAGAACATCACGTGCTGCTACTCTGACCTGTGCAATGTCAACGGGGCCACACCCCTG
 TTCTTCTGTAGTGCACGACGATGAGACTGGACACGTTACAGTTGCCCGGGTGTGGGAC 300
 K K N I T C C Y S D L C N V N G A H T L
 301 AAGCCACCCACACCCCTGGGGCTGCTGACCGTGCTCTGCAGCCTGTTGCTGTGGGGCTCC
 TTCGGTGGGTGGTGGGACCCCGACGACTGGCACGAGACGTCCGACAACGACACCCCGAGG 360
 K P P T T L G L L T V L C S L L L W G S
 361 AGCCGTCTGTAGGCTCTGGGAGAGCCTACCATAGCCCGATTGTGAAGGGATGAGCTGCAC
 TCGGCAGACATCCGAGACCCTCTCGGATGGTATCGGGCTAACACTTCCTACTCGACGTG 420
 S R L
 421 TCCACCCACCCACACAGG 441
 AGGTGGGGTGGGGTGTGTCC

FIGURE 2

1 M K I F L P V T T R A X L N G V S R A S S hSCA-2
 1 M K A V L L A T L M A G E A L O P G T A NPSCA
 1 M K T V L L L L L A T Y T A L H P G A A mPSCA

 21 L M C F S C L N Q K S N L Y C E K P T I
 21 L L C Y S C K A Q V S N E D C L Q V E N
 21 L O C Y S C T A Q M N N R D C L N V Q N

 41 C S D Q D N Y C V T V S A S X G I G N L
 41 C T O L G E Q C W T A R I R A V G L L T
 41 C S L O Q H S C F T S R I R A I G L V T

 61 V T F G H S L S K T C S P A C P I P E G
 61 V - - - - I S K G C S L N C V D D S Q
 61 V - - - - I S K G C S S Q C E D D S E

 81 V N V G V A S H G I S C C Q S F L C N F
 76 D Y Y V G K K - N L T C C O T D L C N A
 76 N Y Y L G K K - N L T C C Y S D L C N V

 101 S A A D G G L R A S V T F G A G G L L
 95 S G A H A L O P A A A L L A L L P A E G
 95 N G A E T L K P P T T L G G D T V E C S

 121 S L L P A L L R E G P
 115 L L L N G P G Q L - -
 115 L L L N G S S L - -

FIGURE 3

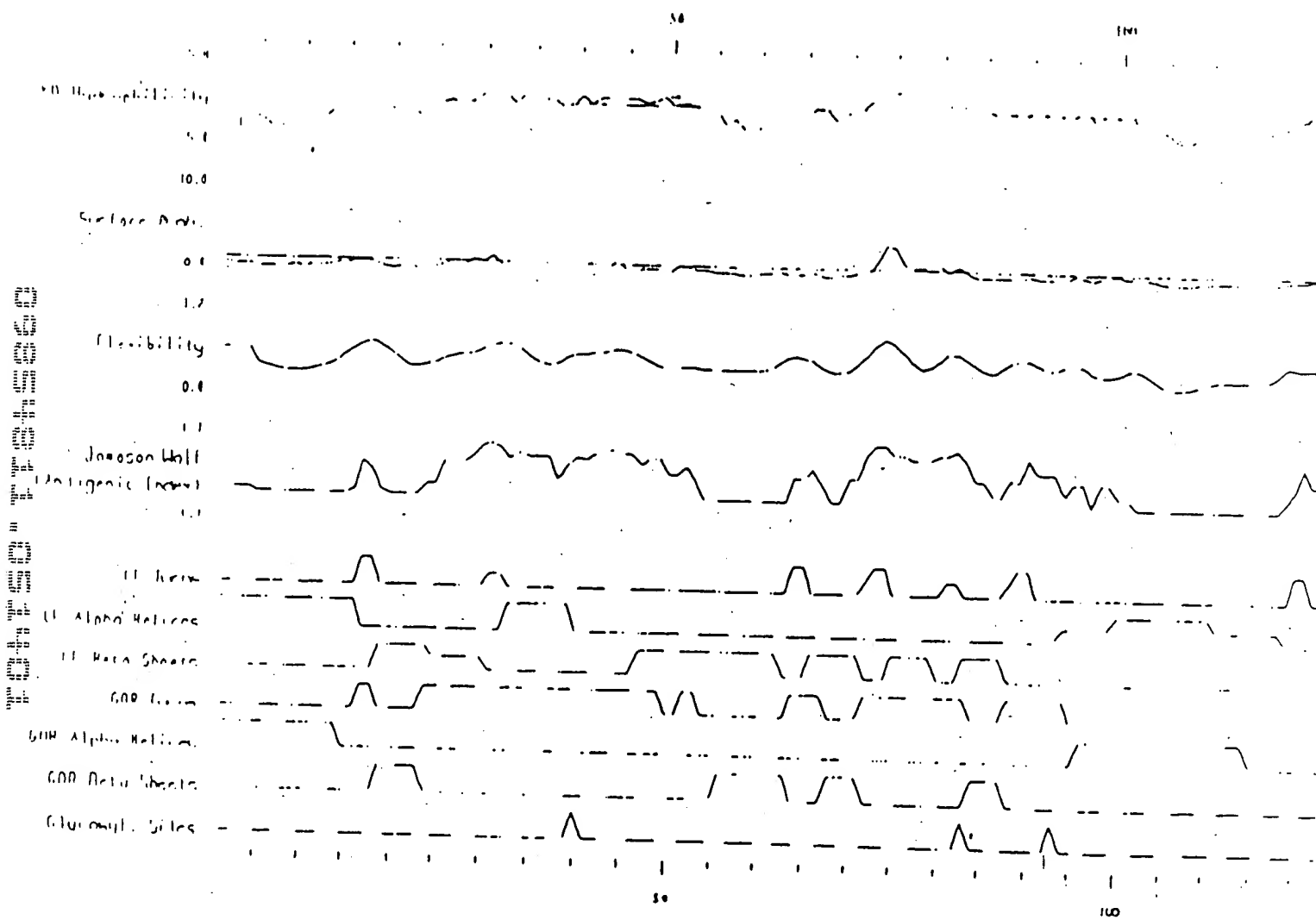


FIGURE 4

↑
signal
sequence

✓ = glycosylation
site

✓

✓ GPI signal

FIGURE 5

Western ASCA
 supposed to be 80% AB
 Normal tissue
 1hr exp

1G8
 1:100

prostate (Kumar)
 prostate (Baker)
 prostate (Glick)
 Bladder (Kumar)
 Bladder (Glick)
 Bladder (Kob)
 Kidney (N104)
 Kidney (WU2)
 Testis
 Sm. Intest.

LA PC9

FIGURE 6

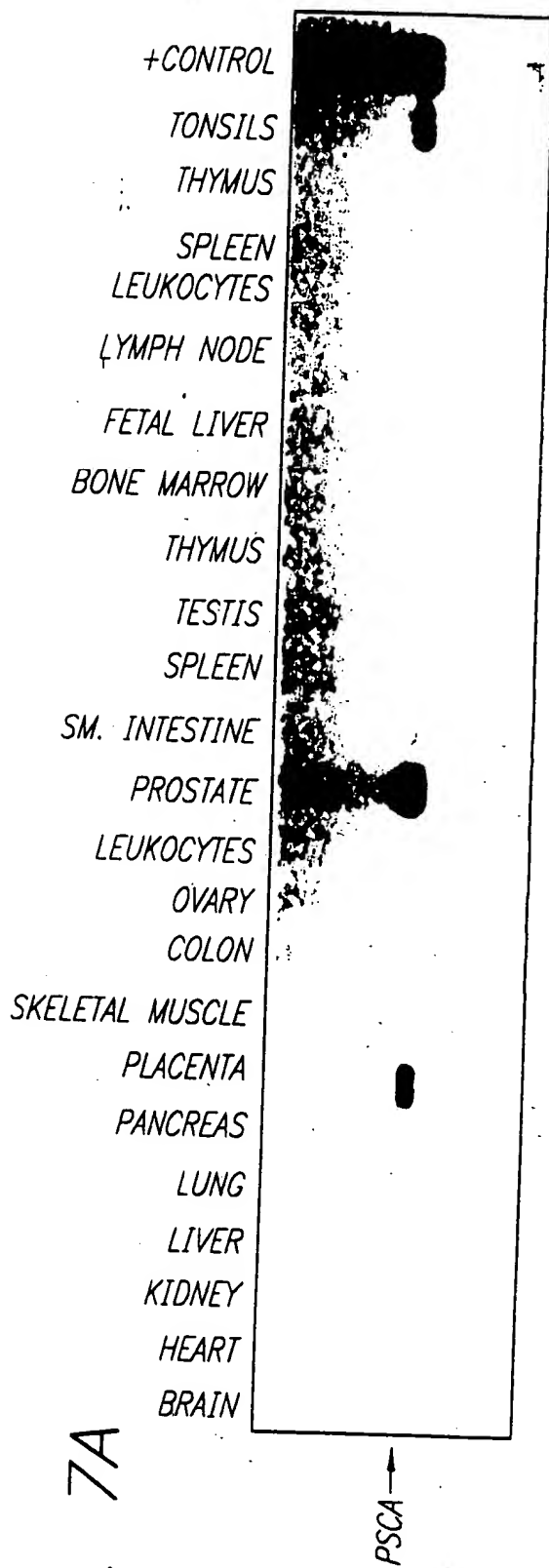


FIG. 7A

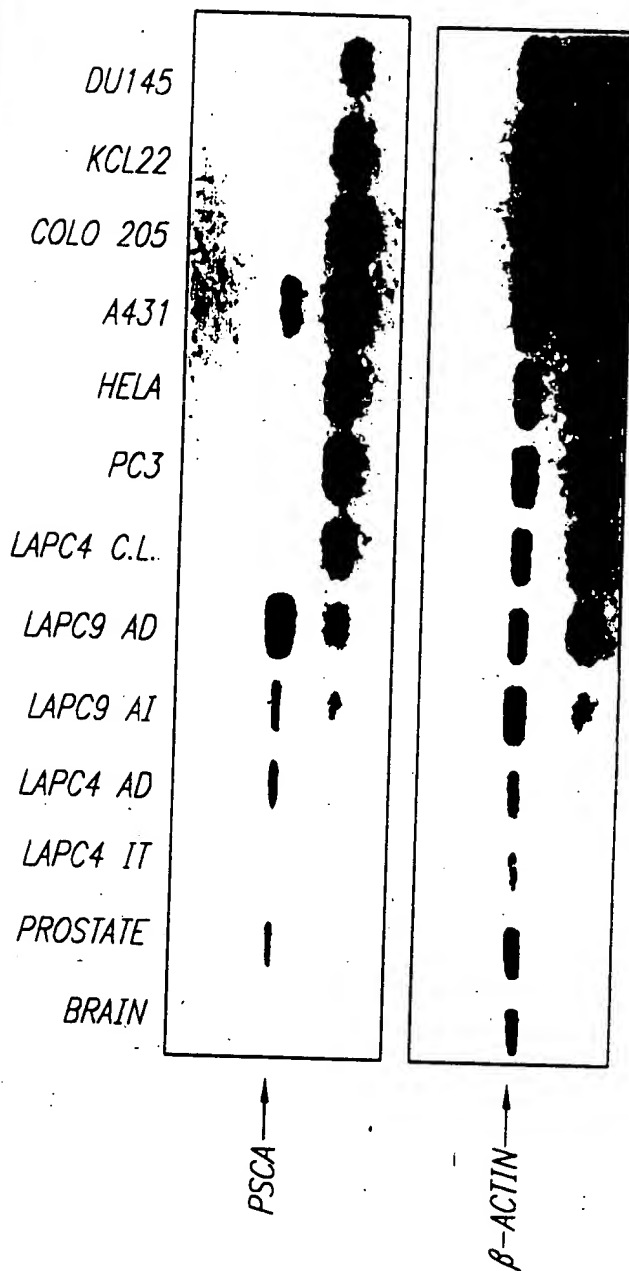



FIG. 7B

Legend:  untranslated region of pSCA

 translated region of pSCA

246 bp

FIG. 8A

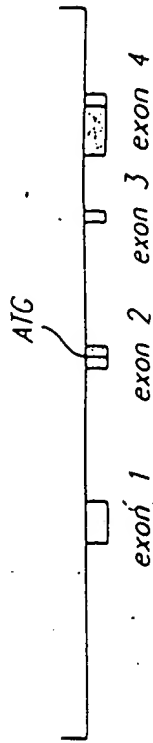


FIG. 8B

murine pSCA

FIGURE 8

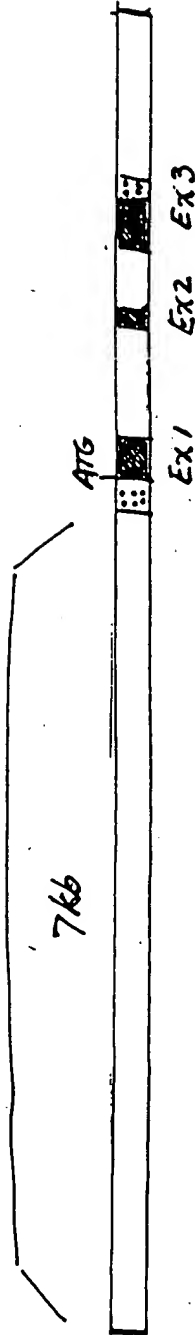
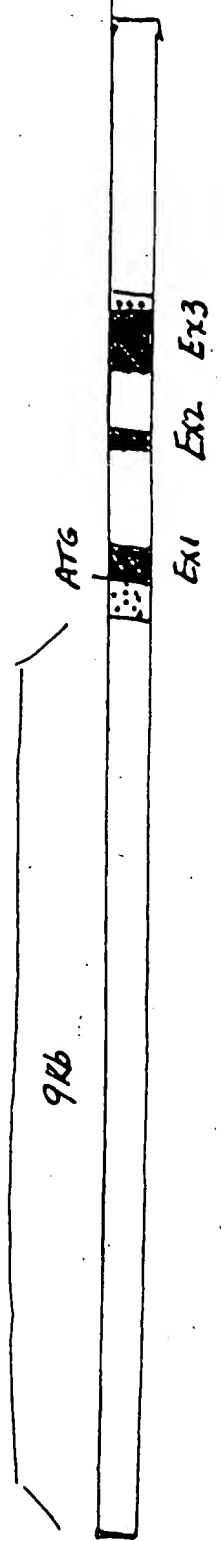


FIG. 8C

human pSCA



PSCA / PSA Expression in Benign Prostate vs. Prostate Cancer Xenograft

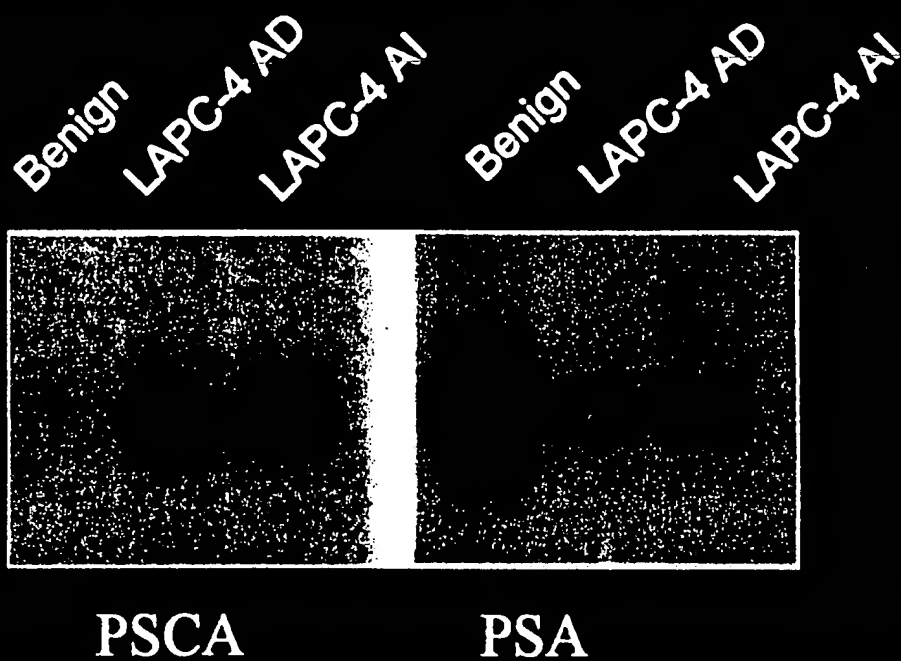


FIGURE 9A

~ 1kb

PANCREAS
KIDNEY
SKELETAL MUSCLE
LIVER
LUNG
PLACENTA
BRAIN
HEART



PERIPHERAL LEUKOCYTES
COLON
SMALL INTESTINE
OVARY
TESTIS
PROSTATE
THYMUS
SPLEEN



PSCA

FIG. 9B

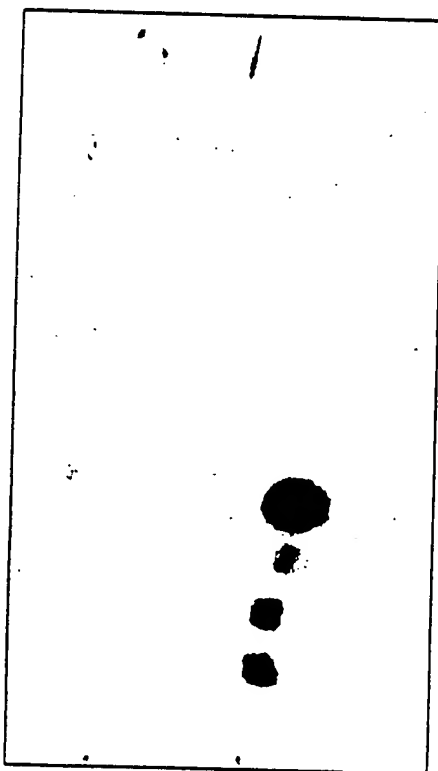
72 HRS

KCL22
 COLO 205
 A431
 HELA
 DU145
 PC3
 LNCAP
 LAPC4 C.L.
 LAPC3 AI
 LAPC9
 LAPC4 IT
 LAPC4 AI
 LAPC4 AD
 BPH



4 HRS

KCL22
 COLO 205
 A431
 HELA
 DU145
 PC3
 LNCAP
 LAPC4 C.L.
 LAPC3 AI
 LAPC9
 LAPC4 IT
 LAPC4 AI
 LAPC4 AD
 BPH



PSCA

FIG. 10-1

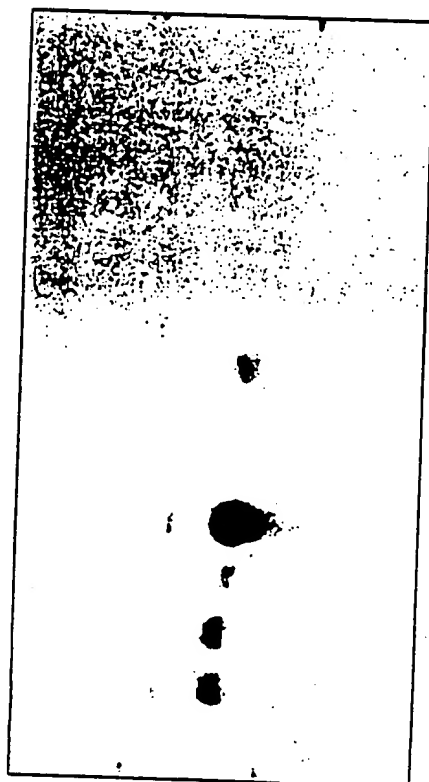
72 HRS

KCL22
COLO 205
A431
HELA
DU145
PC3
LNCAP
LAPC4 C.L.
LAPC3 AI
LAPC9
LAPC4 IT
LAPC4 AI
LAPC4 AD
BPH



4 HRS

KCL22
COLO 205
A431
HELA
DU145
PC3
LNCAP
LAPC4 C.L.
LAPC3 AI
LAPC9
LAPC4 IT
LAPC4 AI
LAPC4 AD
BPH



MSA

FIG. 10-2

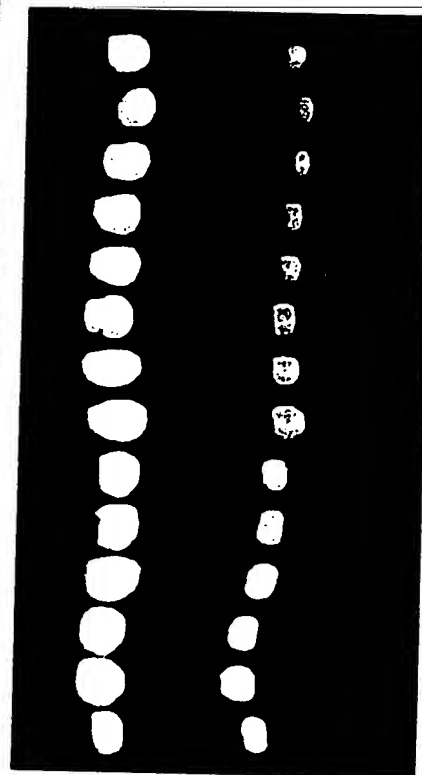
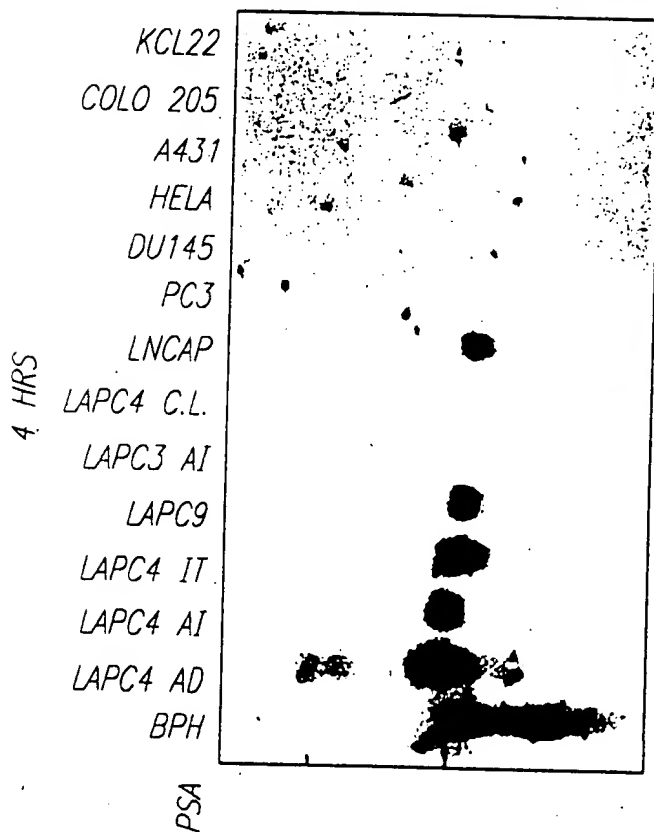
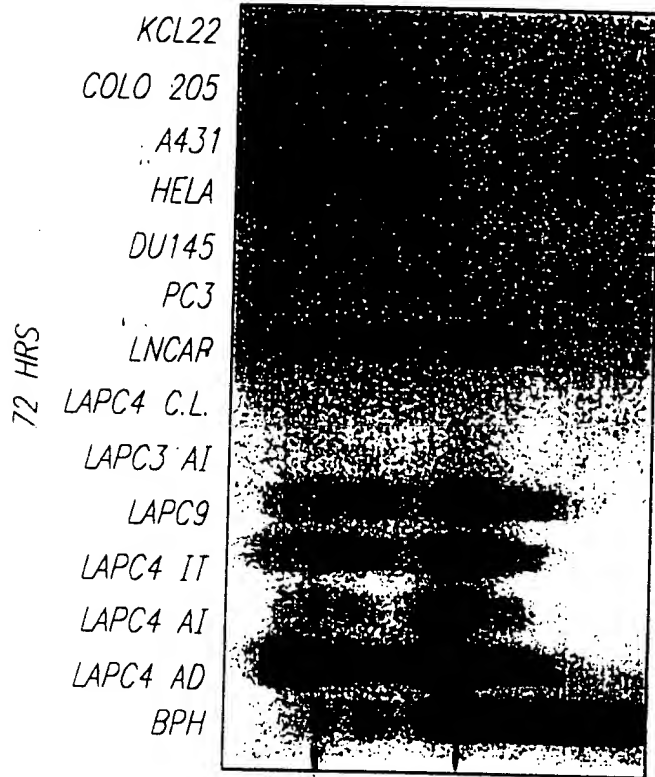


FIG. 10-3

FIG. 11A



FIG. 11B

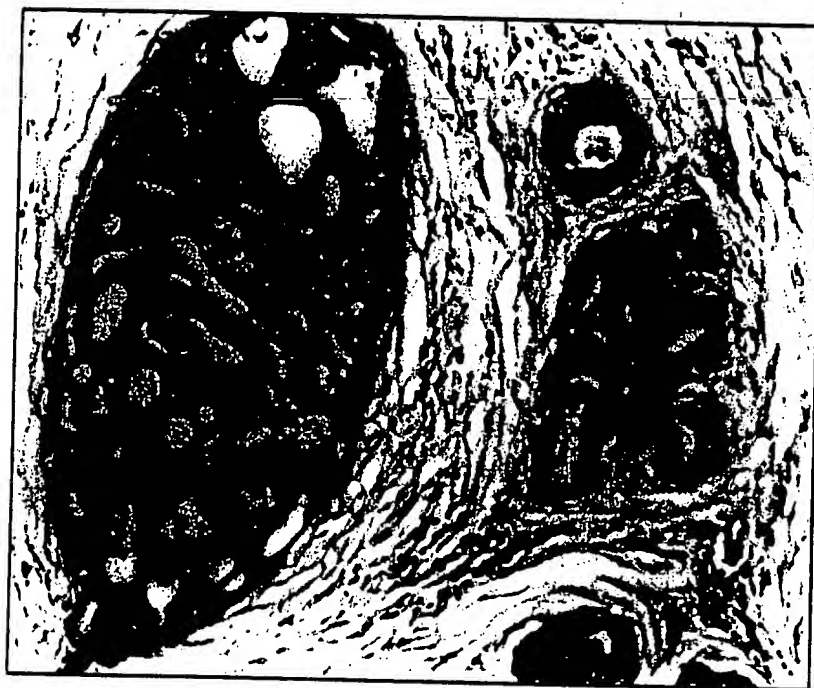
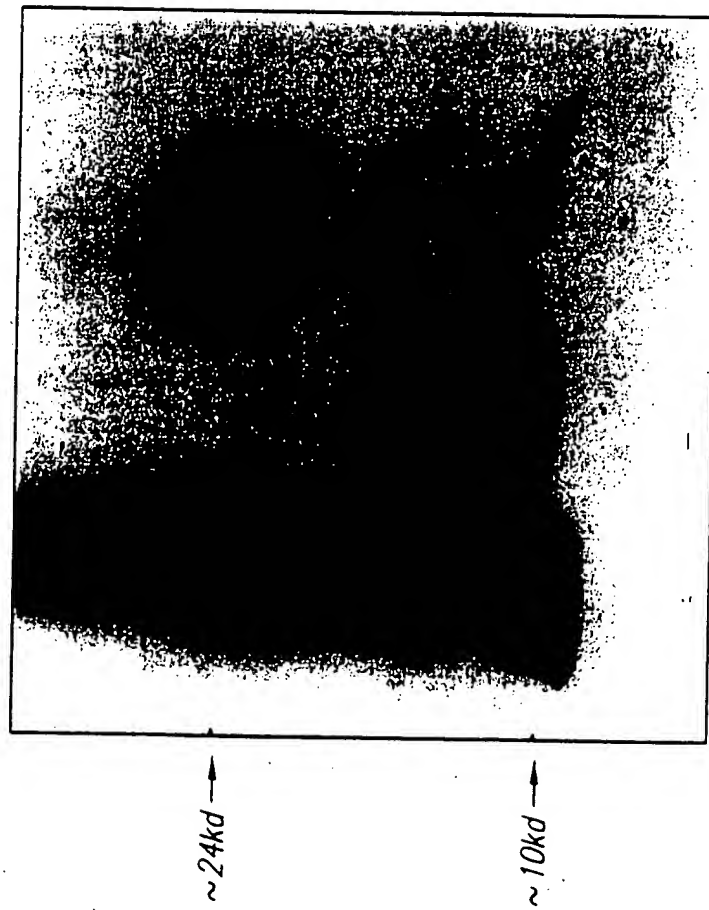


FIG. 11C

FIG. 12A

O GLYCOSIDASE
N GLYCOSIDASE F
CONTROL



SECRETED
CELL ASSOCIATED

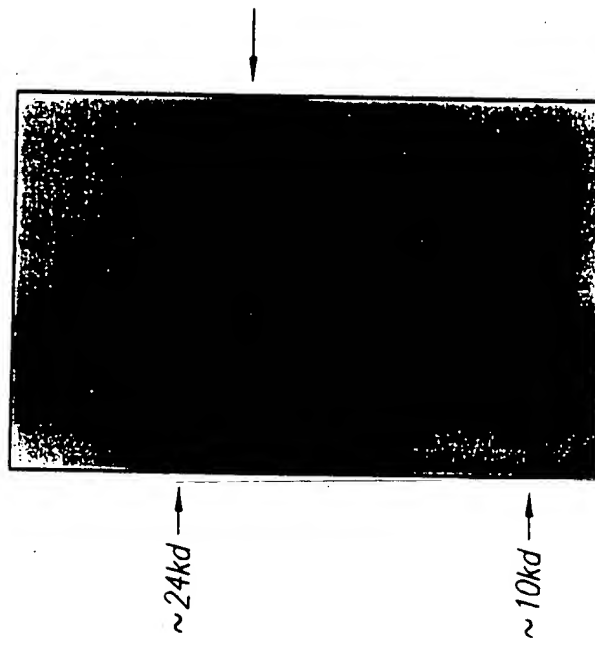


FIG. 12B

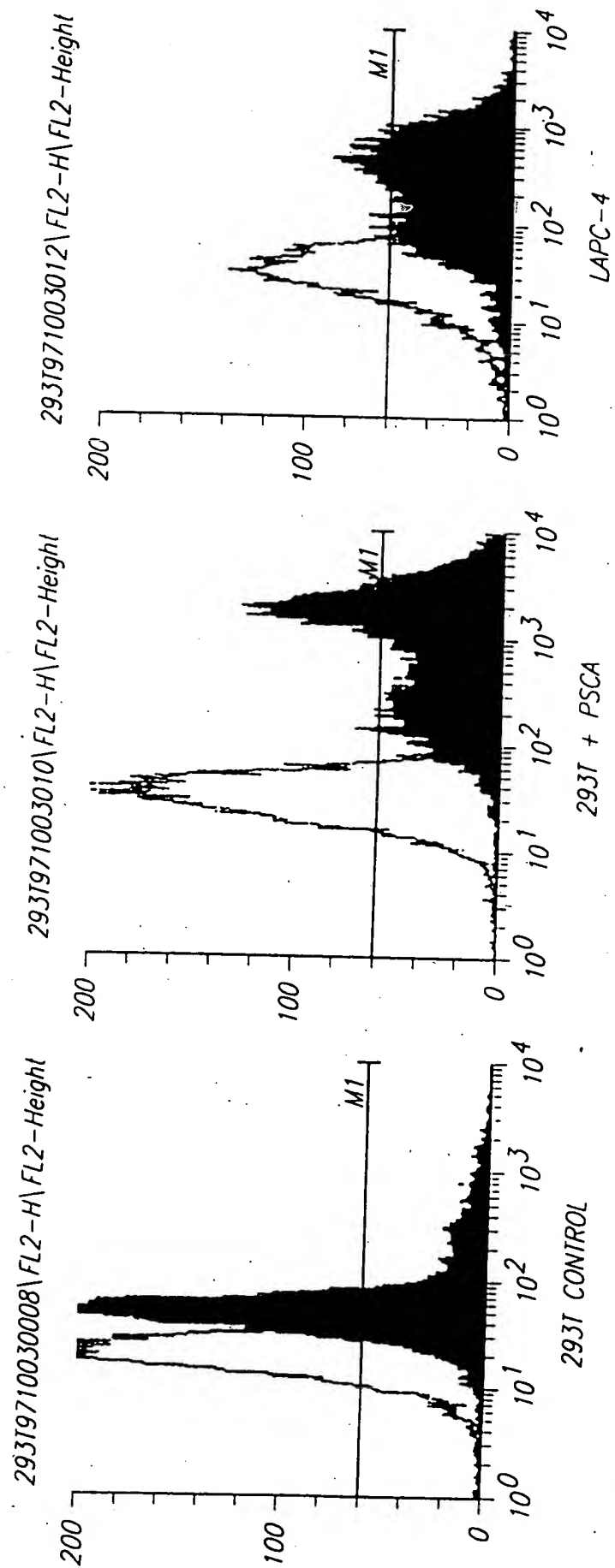
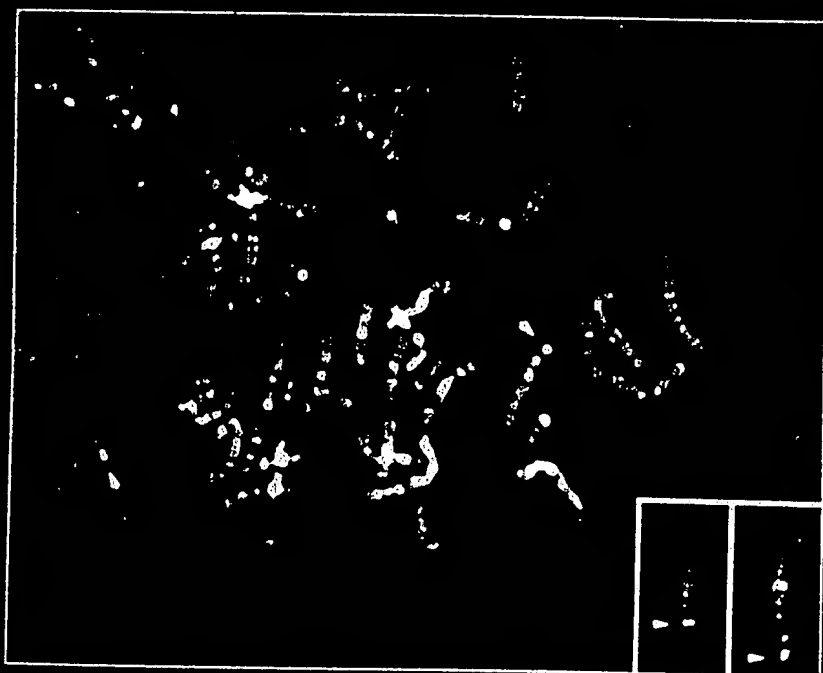


FIGURE 12C

PSCA Maps to Chromosome 8q24.2



Fluorescent
in Situ Hybridization
Analysis of PSCA

FIGURE 13

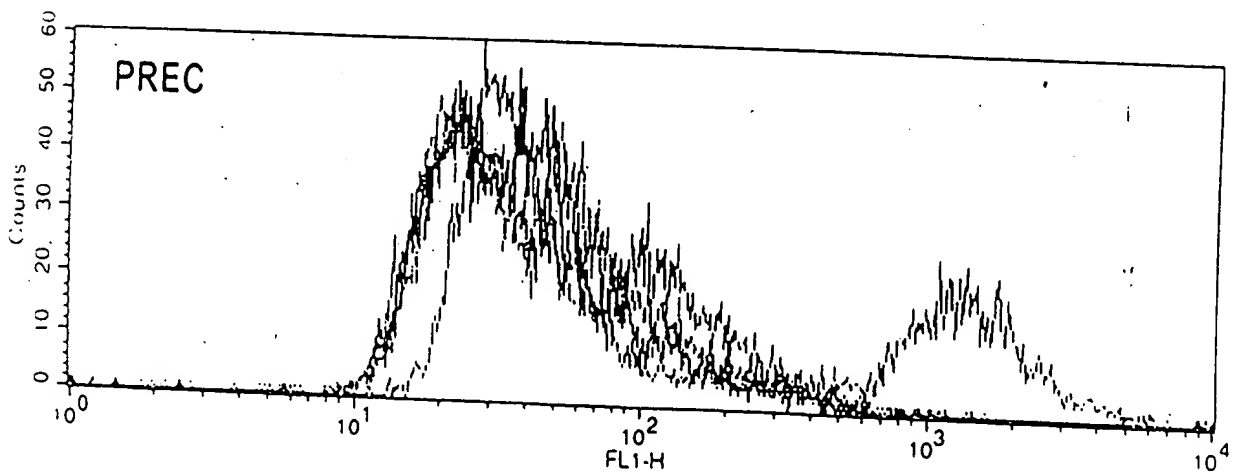
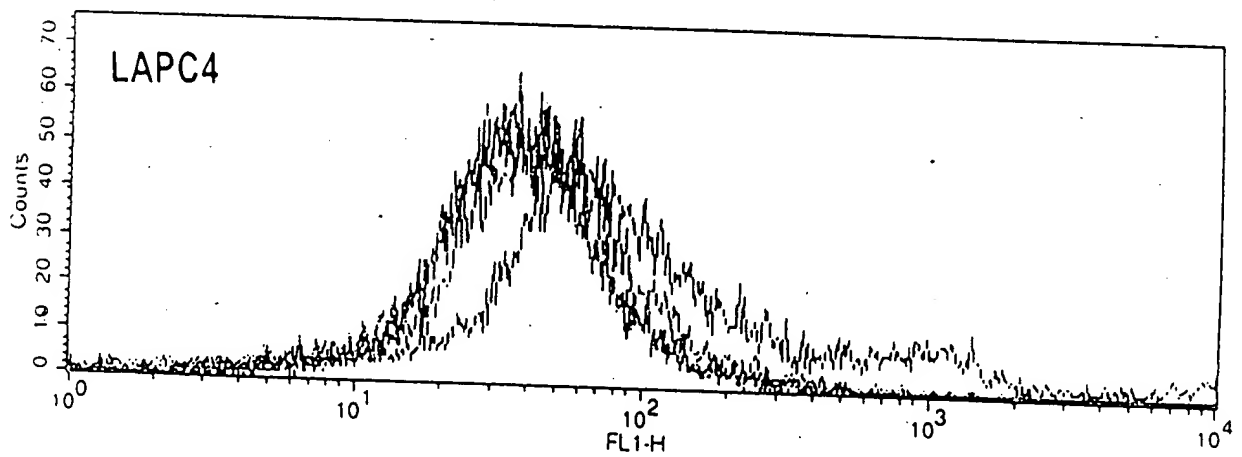
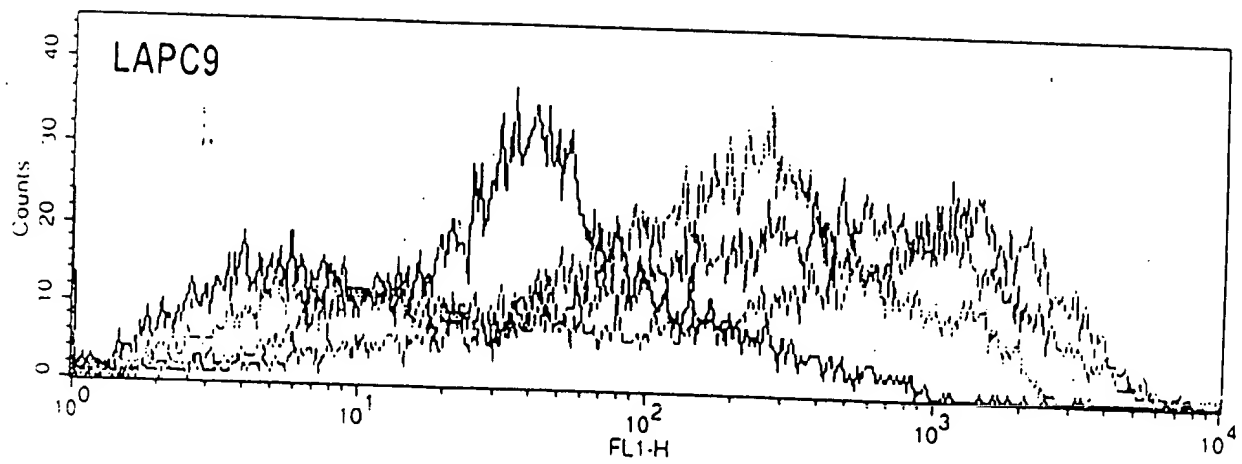


FIGURE 14

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

A

Epitope map

mAb	Isotype	EL (18-98)	N (2-50)	M (46-109)	C (85-123)
1G8	IgG1 k	2.039	0.007	0.628	0.000
2H9	IgG1 k	1.318	0.863	0.032	0.021
3C5	IgG2a k	2.893	1.965	0.016	0.005
3E6	IgG3 k	0.328	0.024	0.069	0.370
4A10	IgG2a k	2.039	1.315	0.000	0.014
2A2	IgG2a k	1.366	0.733	0.010	0.003
3G3	IgG2a k	2.805	1.731	0.004	0.000

B

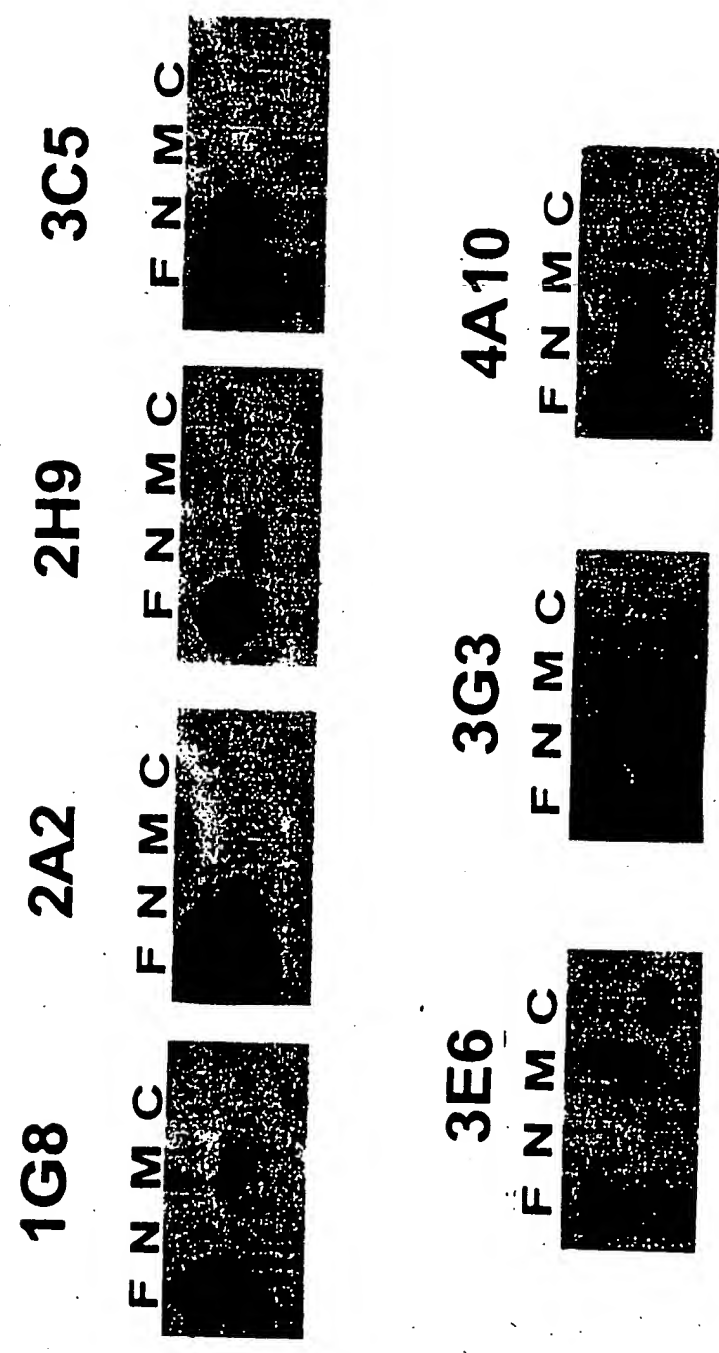
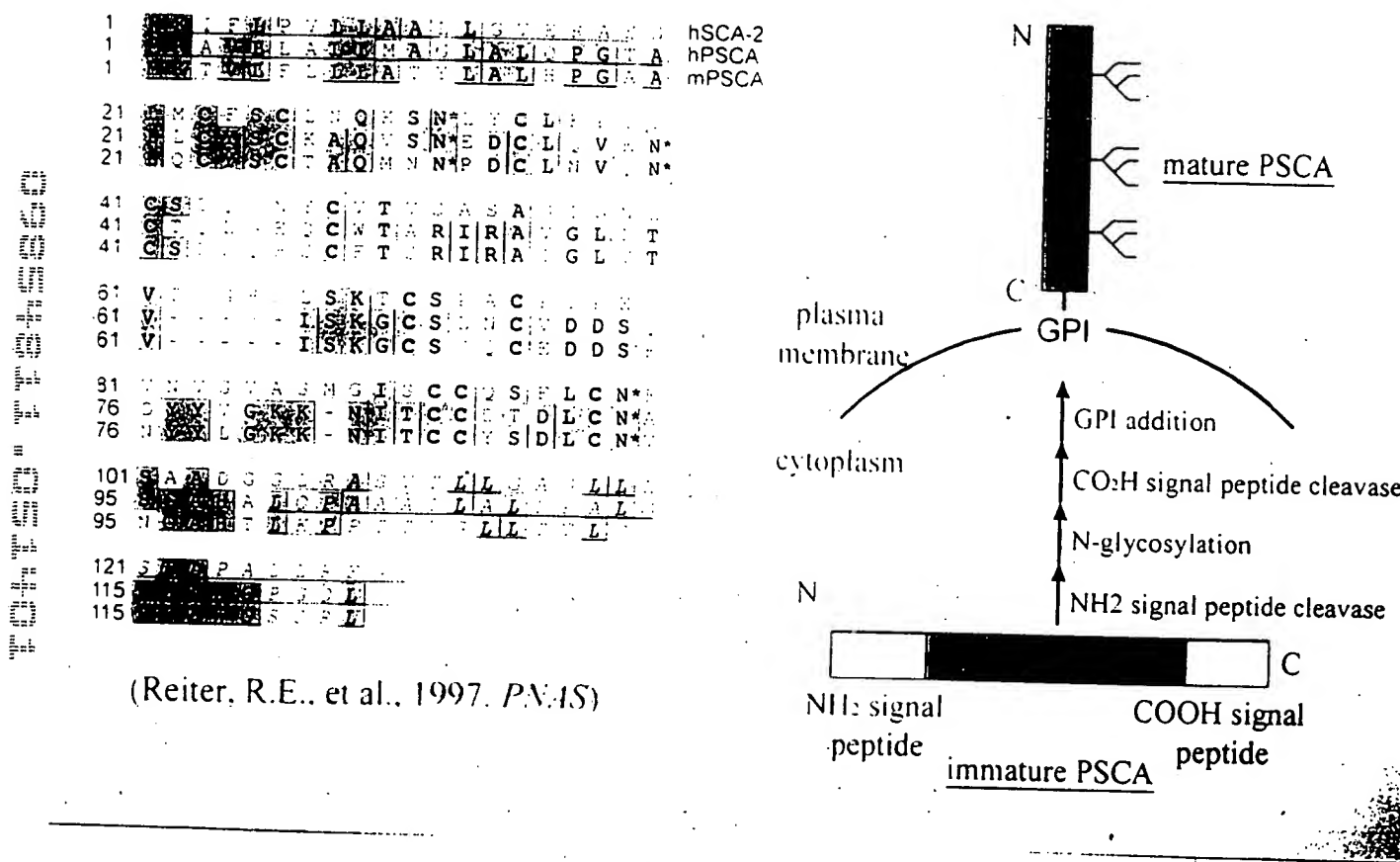


FIGURE 15

Prostate Stem Cell Antigen (PSCA) is a GPI-anchored Protein



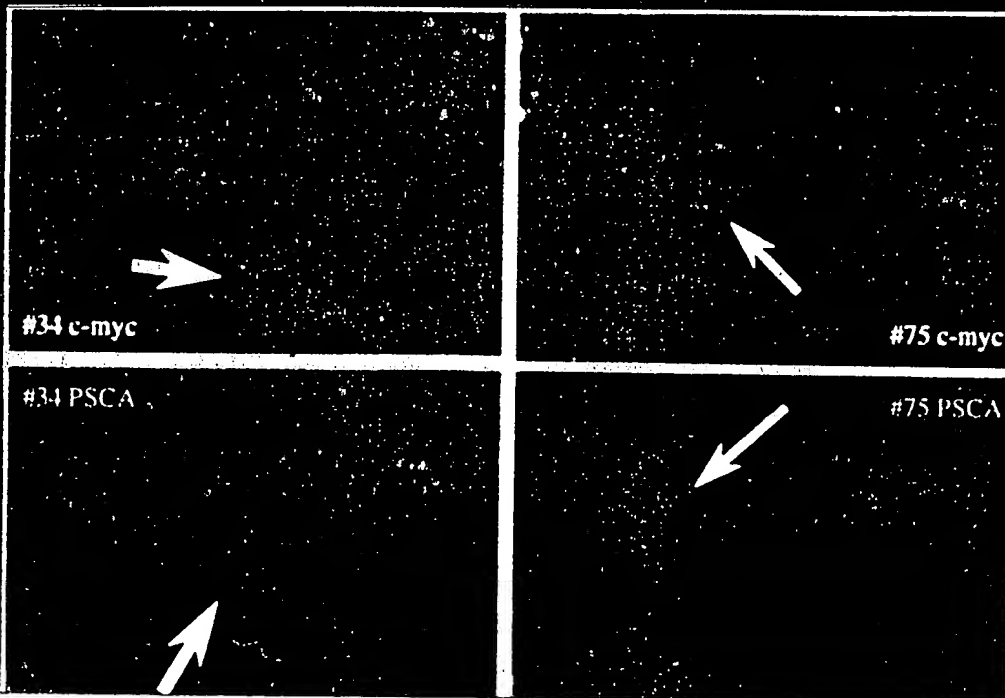
(Reiter, R.E., et al., 1997. *PNAS*)

FIGURE 16

FISH Analysis of PSCA and c-myc in Prostate Cancer

Gain Chromosome 8

Amplification



R. Jenkins

FIGURE 17

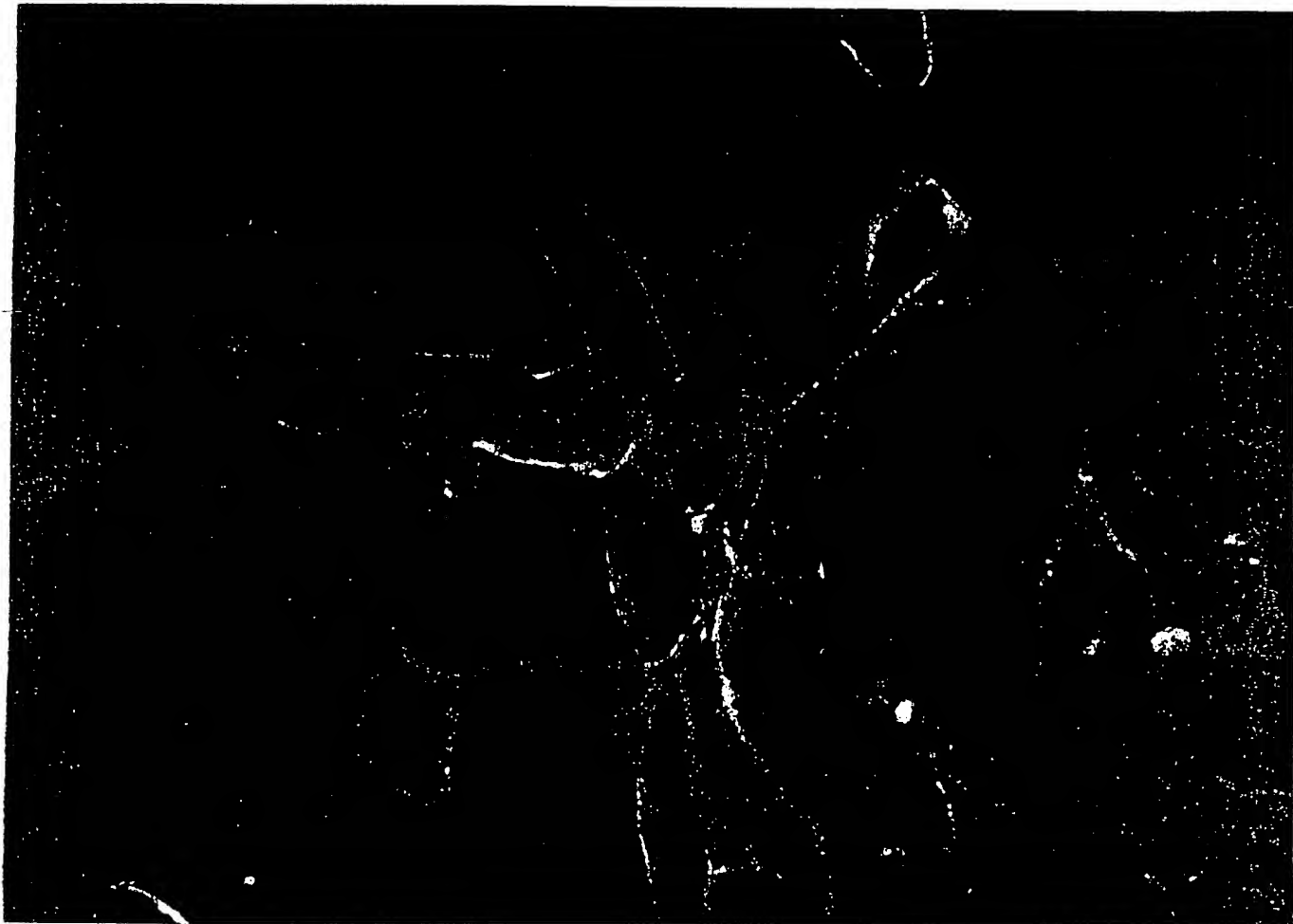


FIGURE 18

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99

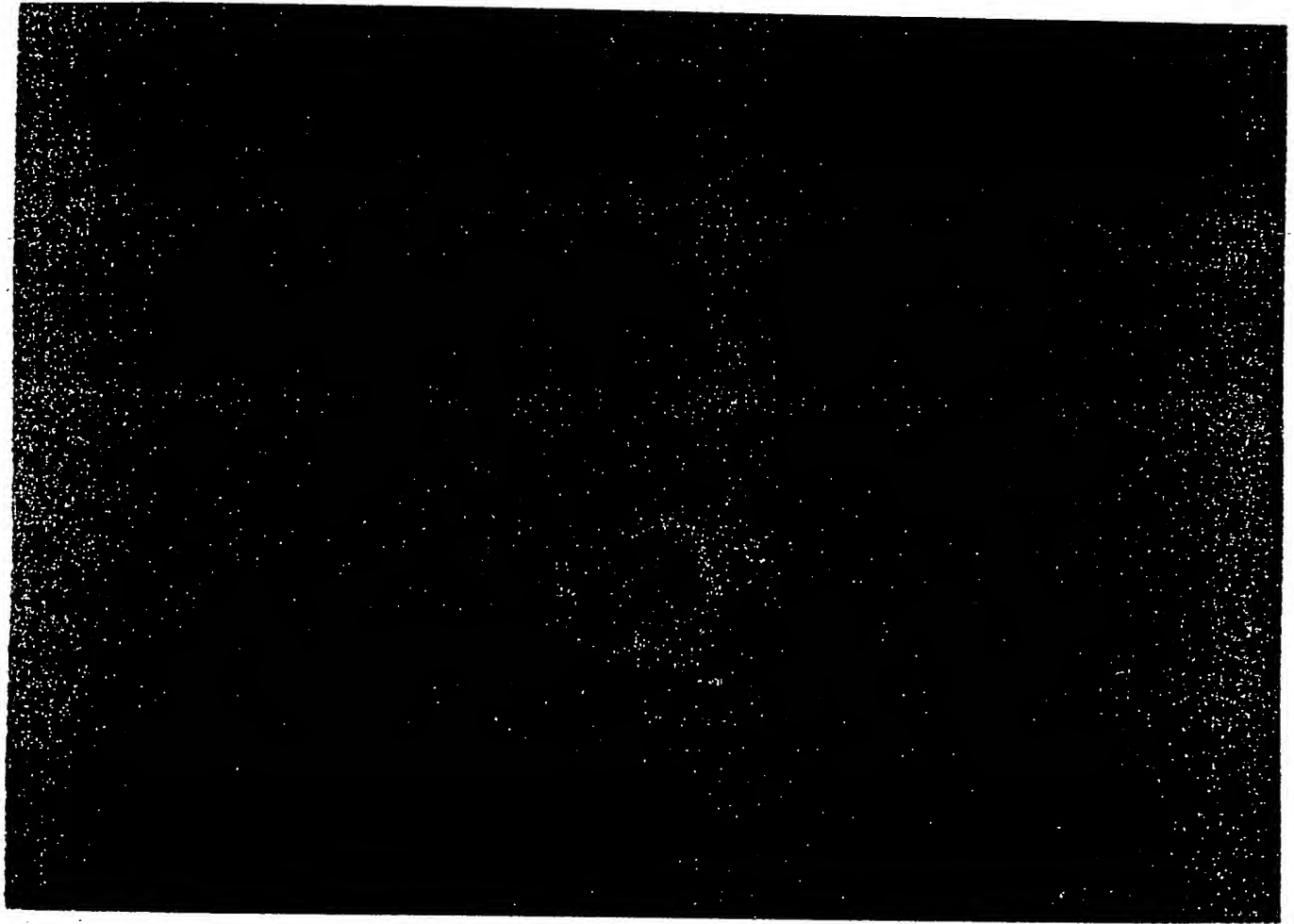


FIGURE 19

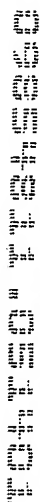


FIGURE 20

PSCA Immunostaining of Primary Tumors

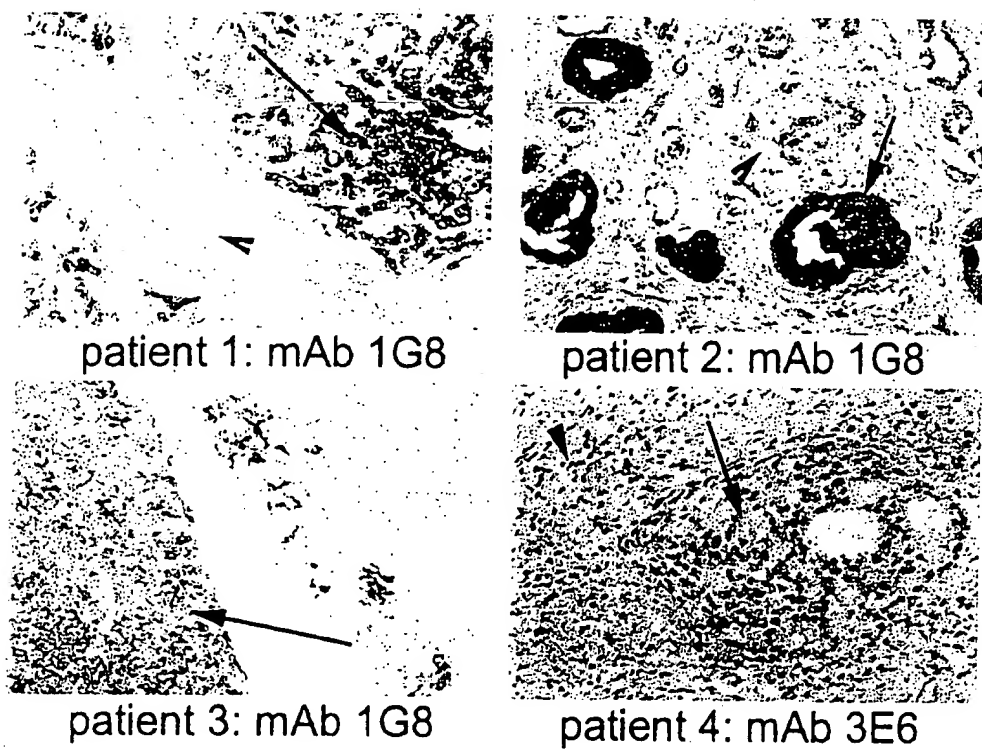


FIGURE 21



FIGURE 22

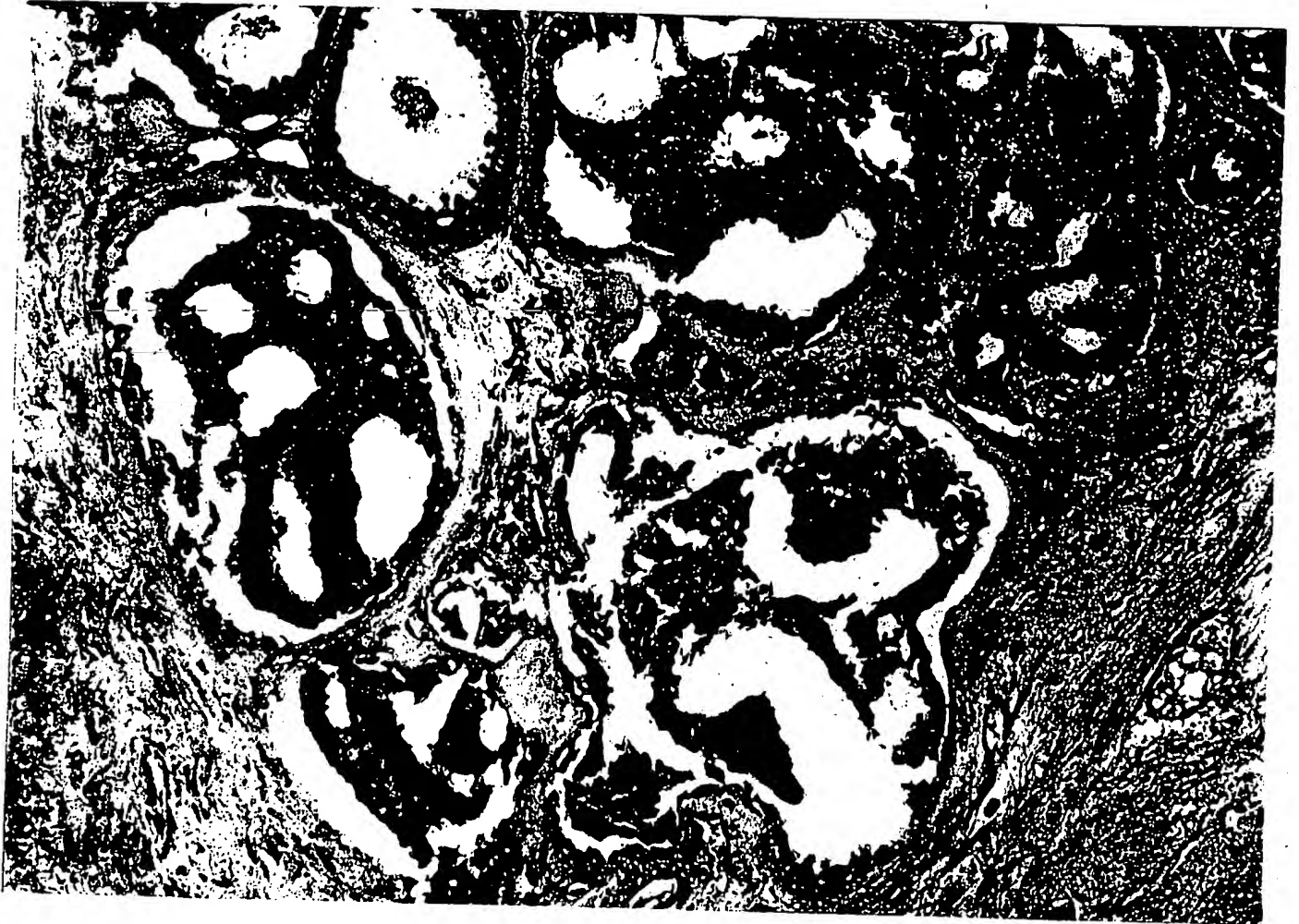


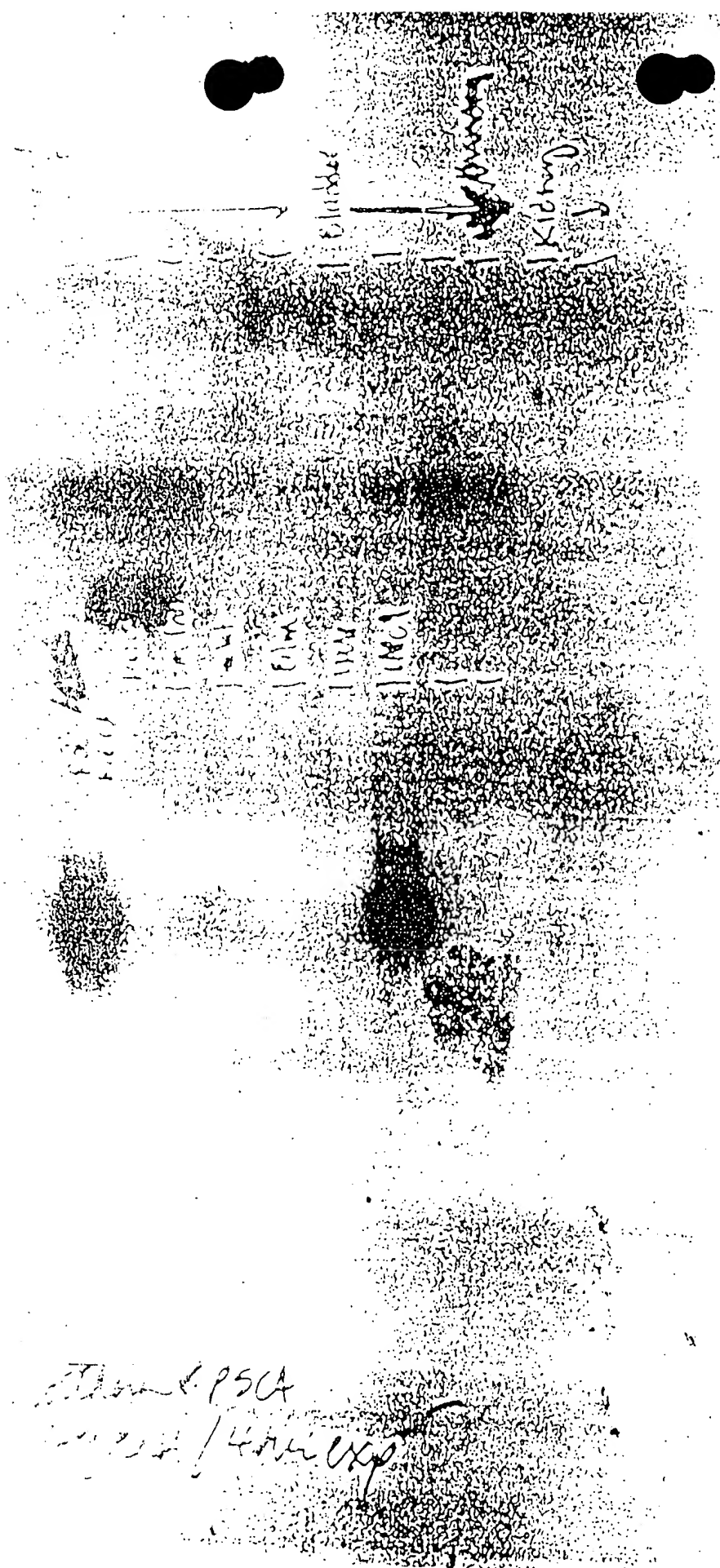
FIGURE 23

0305434 0344034



FIGURE 24

0985434 054434



Thom & PSCA
10/22/14 / 4000 EXP

FIGURE 25

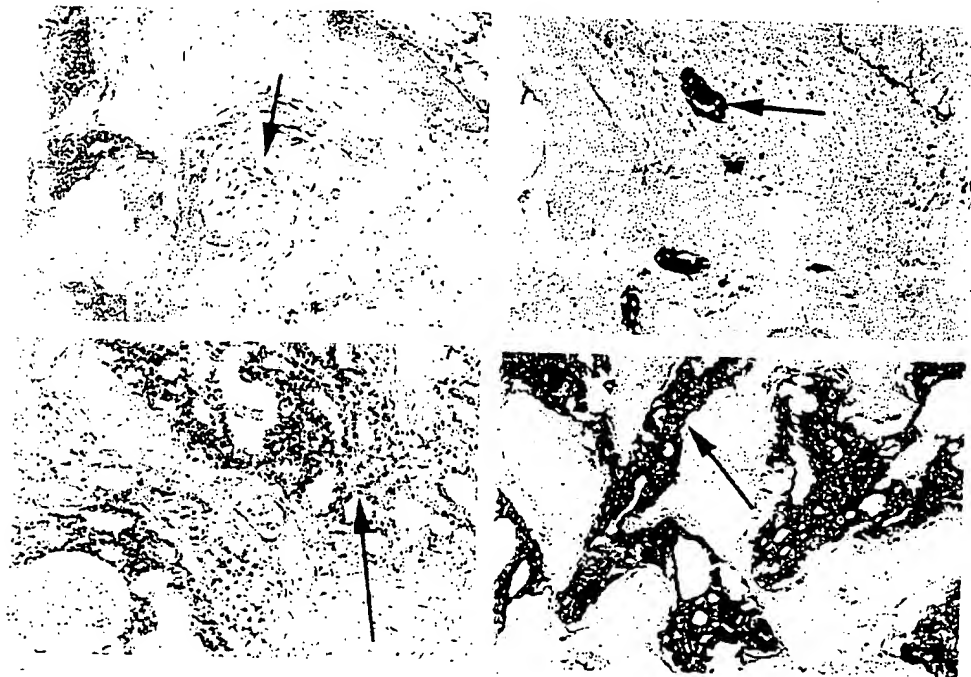


FIGURE 26

A high-contrast, black and white photograph of a large, irregularly shaped, light-colored object, possibly a piece of debris or a biological specimen, resting on a dark, textured surface. The object has a rough, porous appearance with many small holes and indentations. The background is dark and grainy.

FIGURE 27

PSCA Immunostaining of Bony Metastases



Patient 5: H and E
and mAb 1G8

Patient 4: H and E
and mAb 3E6

FIGURE 28



FIGURE 29

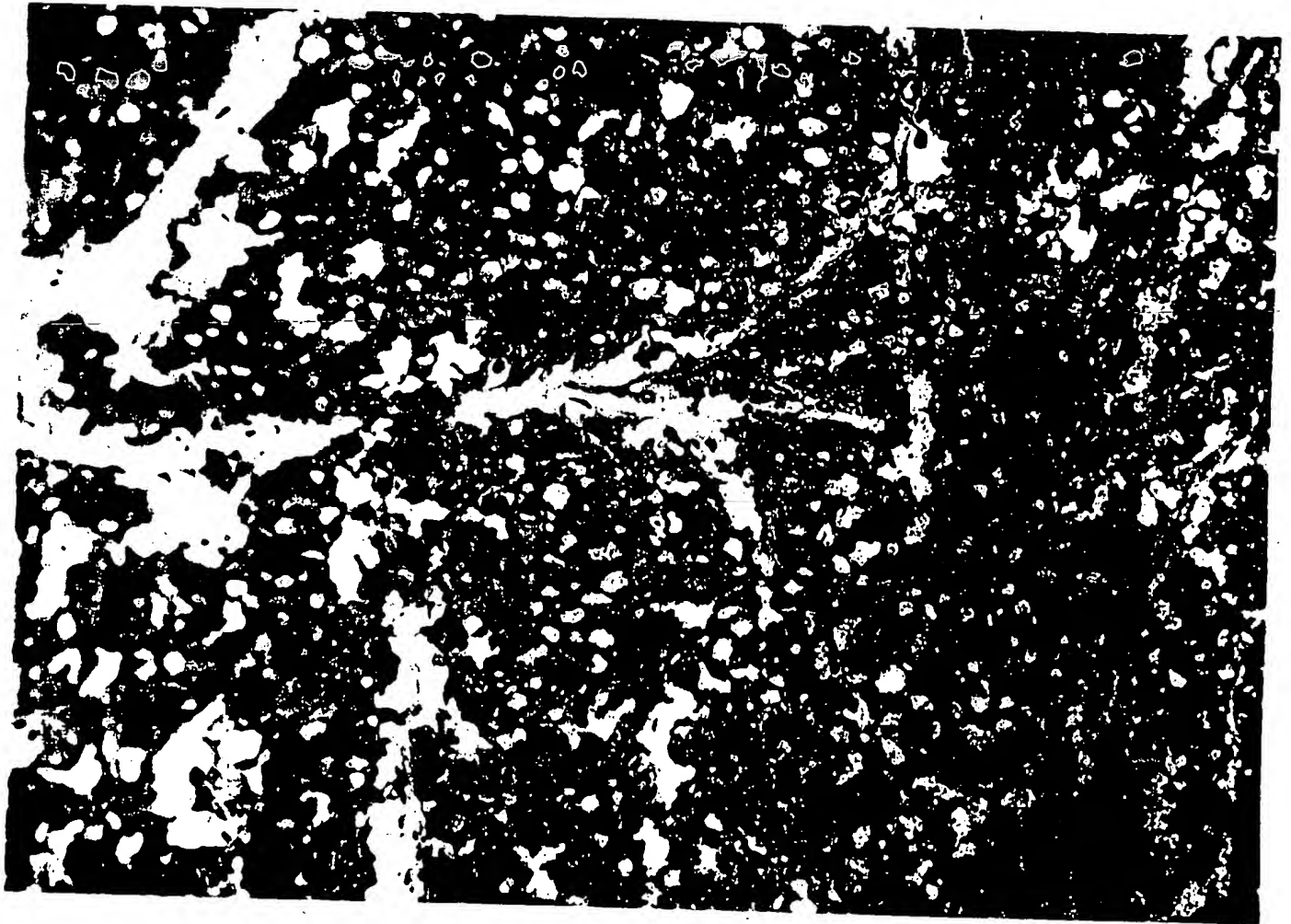


FIGURE 31

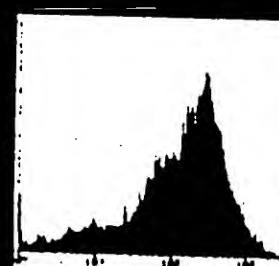
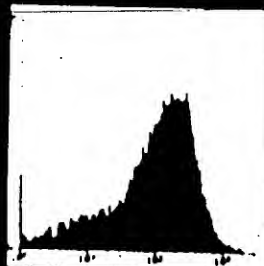
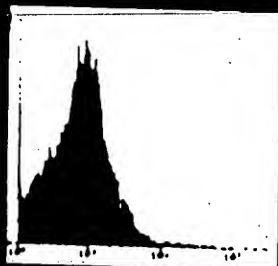
A high-contrast, black and white image showing a dense, textured surface, possibly a rock face or a heavily patterned material. The texture is irregular and granular, with many small, light-colored spots and patches against a dark background. The overall appearance is rough and uneven.

PSCA Expression in LAPC-9 Xenograft by FACS

Secondary Antibody

1G8

2H9



4A10

3C5

3E6

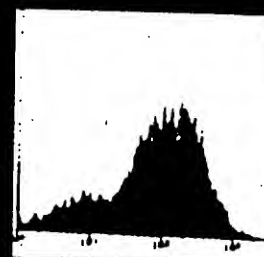
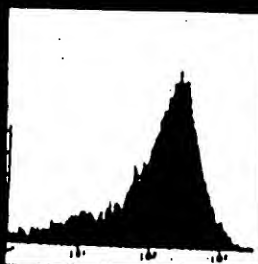
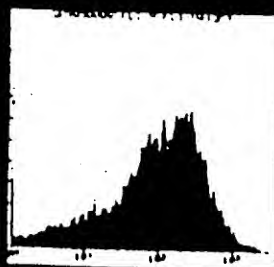


FIGURE 33

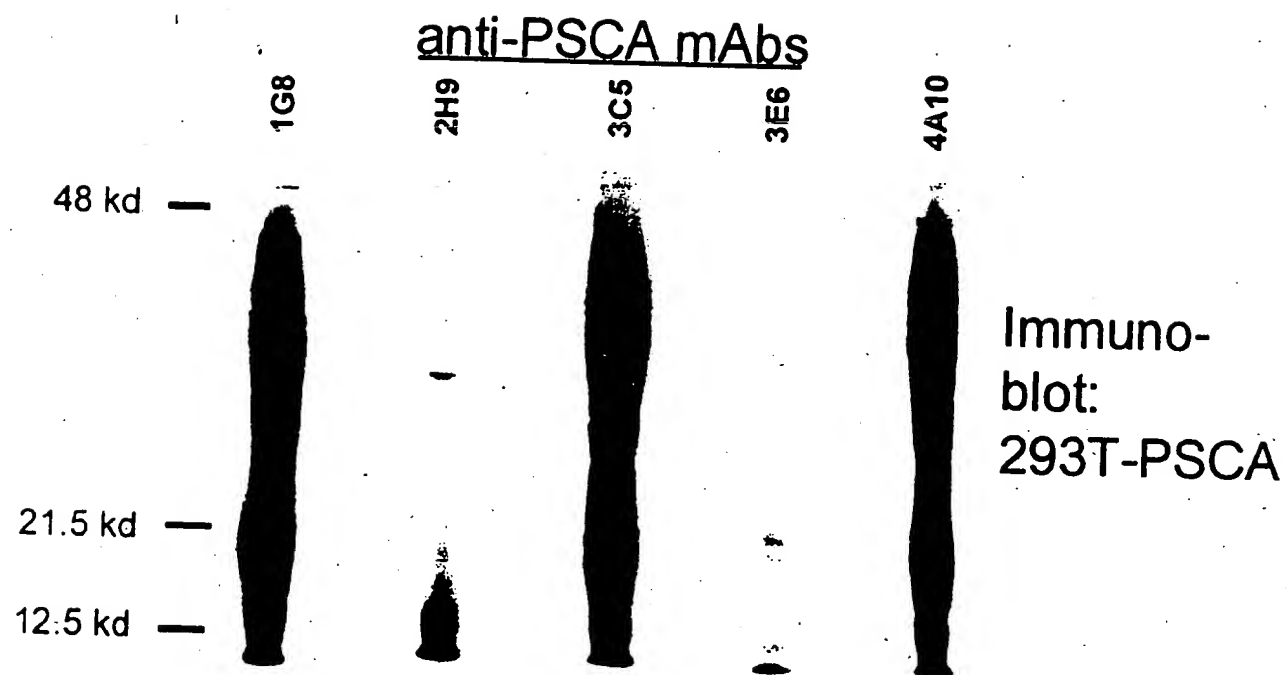


FIGURE 34

Immunofluorescent Staining of LNCaP-PSCA Cells

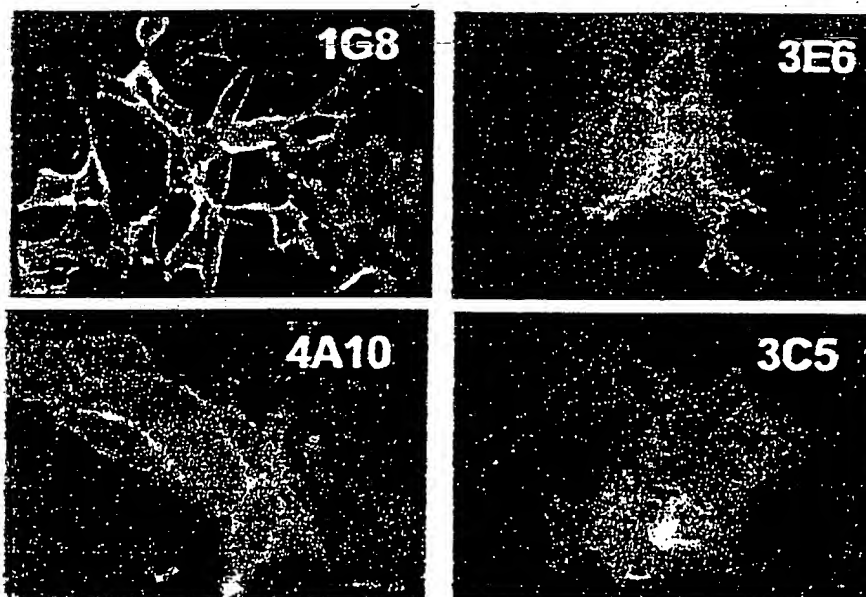


FIGURE 35

FIGURE 36

Immunohistochemical Staining of Normal Prostate

Normal: Isotype Control



Normal: PSCA mAb 3E6



Normal: PSCA mAb 1G8



Atrophy: PSCA mAb 2H9

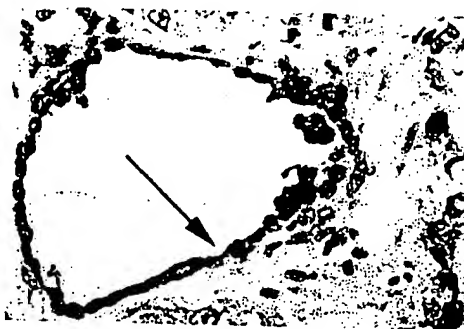
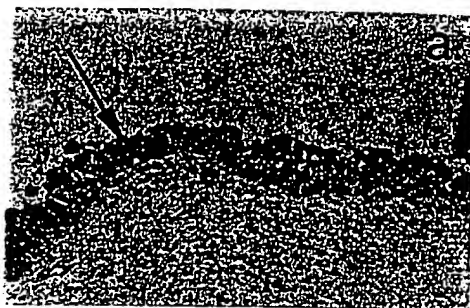
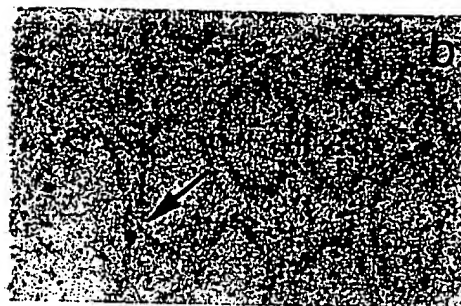


FIGURE 38

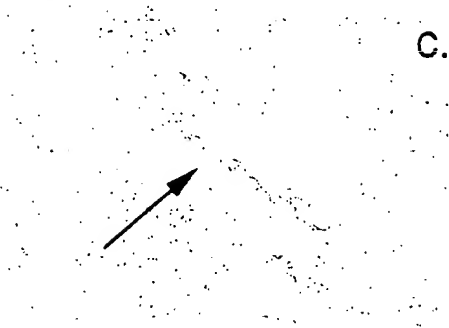
A.



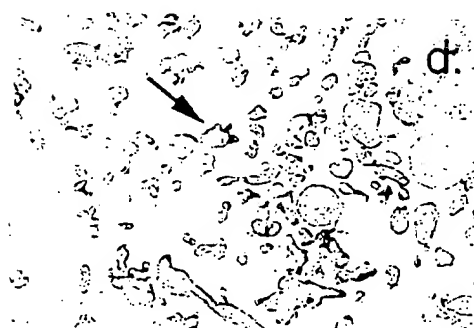
Bladder: 1G8



Colon: 1G8



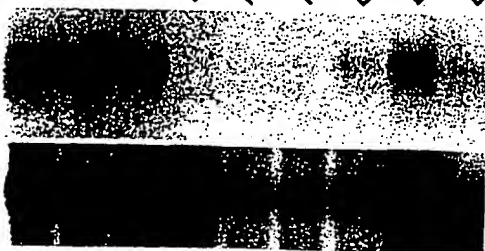
Kidney: 3E6



Placenta: 3E6

B.

Prostate
Prostate
Prostate
Kidney
Kidney
Kidney
Bladder
Bladder
Bladder
LAPC 9

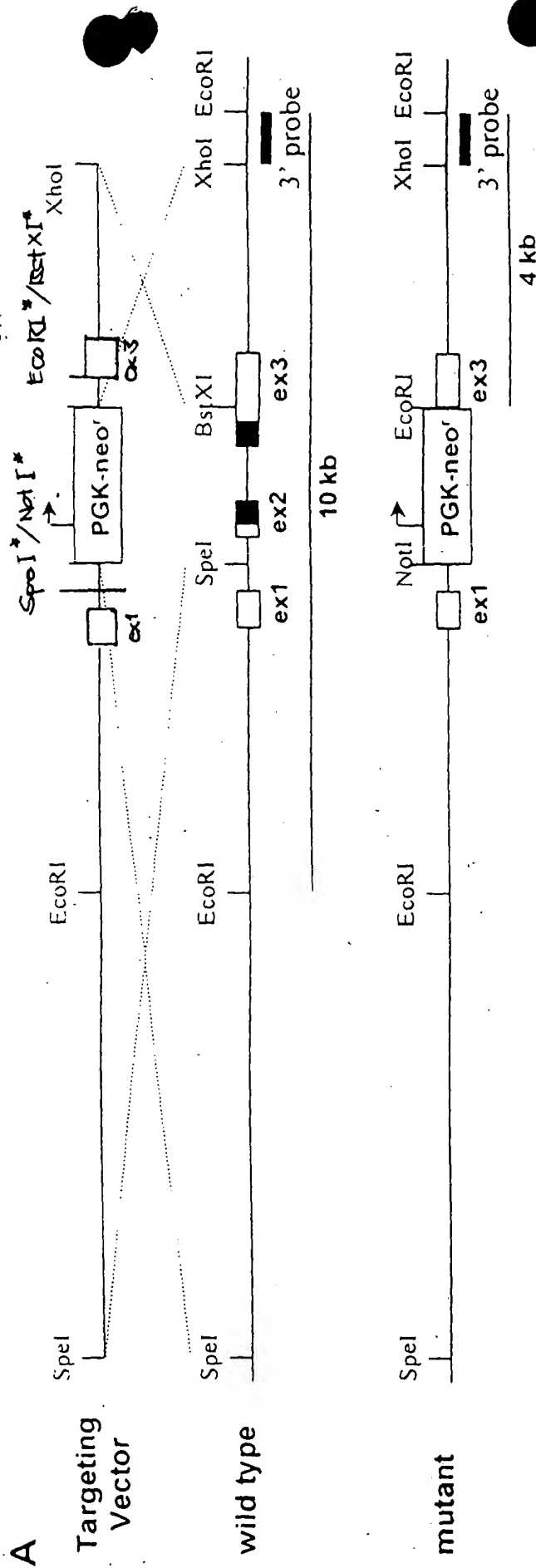


PSCA

Actin

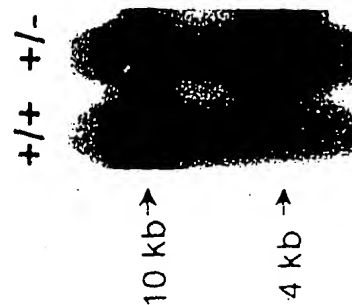
FIGURE 39

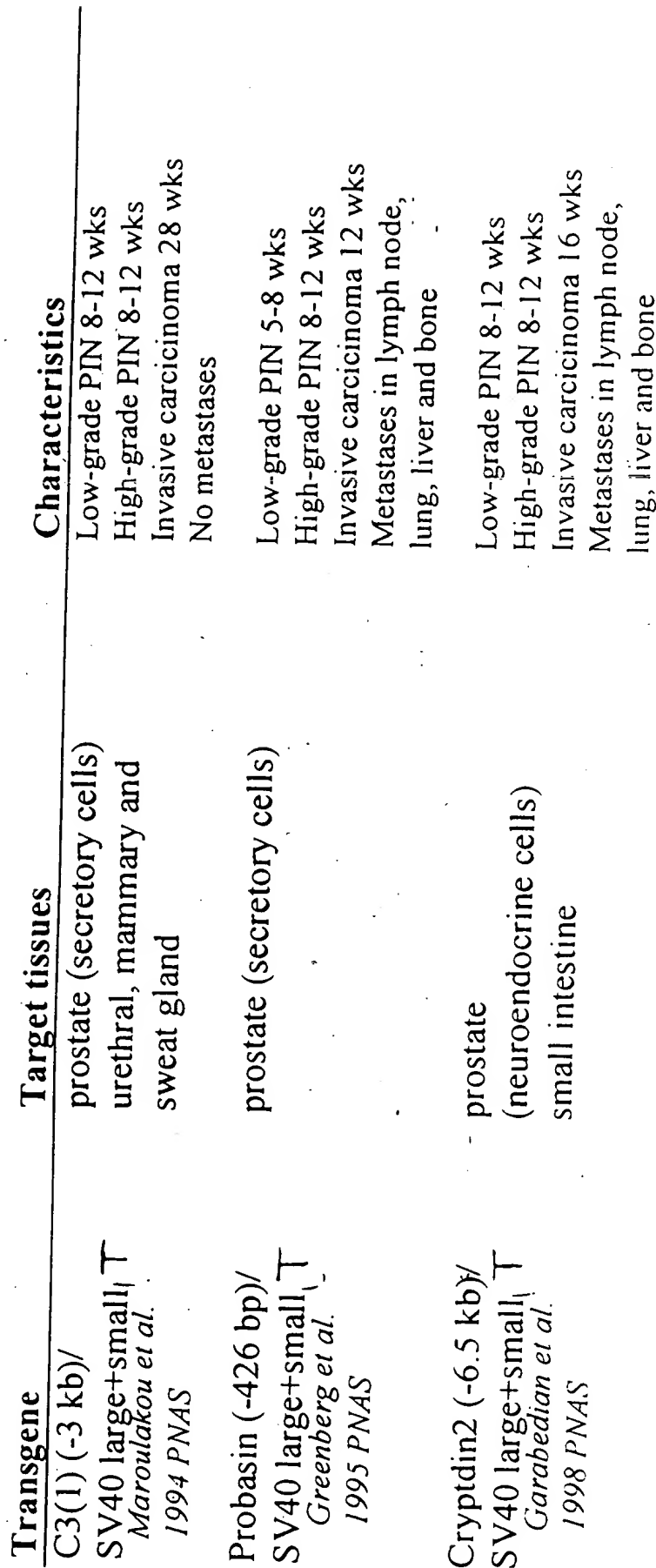
Targeting of Mouse PSCA Gene



B. Genomic Southern Analysis of ES Cells

- * ex1, 2, and 3 are the exons of PSCA gene.
- * Black boxes of ex2 and ex3 encode PSCA mature protein sequences.
- * ES genomic DNAs were digested with EcoRI, followed by Southern hybridization using 3' probe.





Reporter Gene Constructs for Transfection Assay

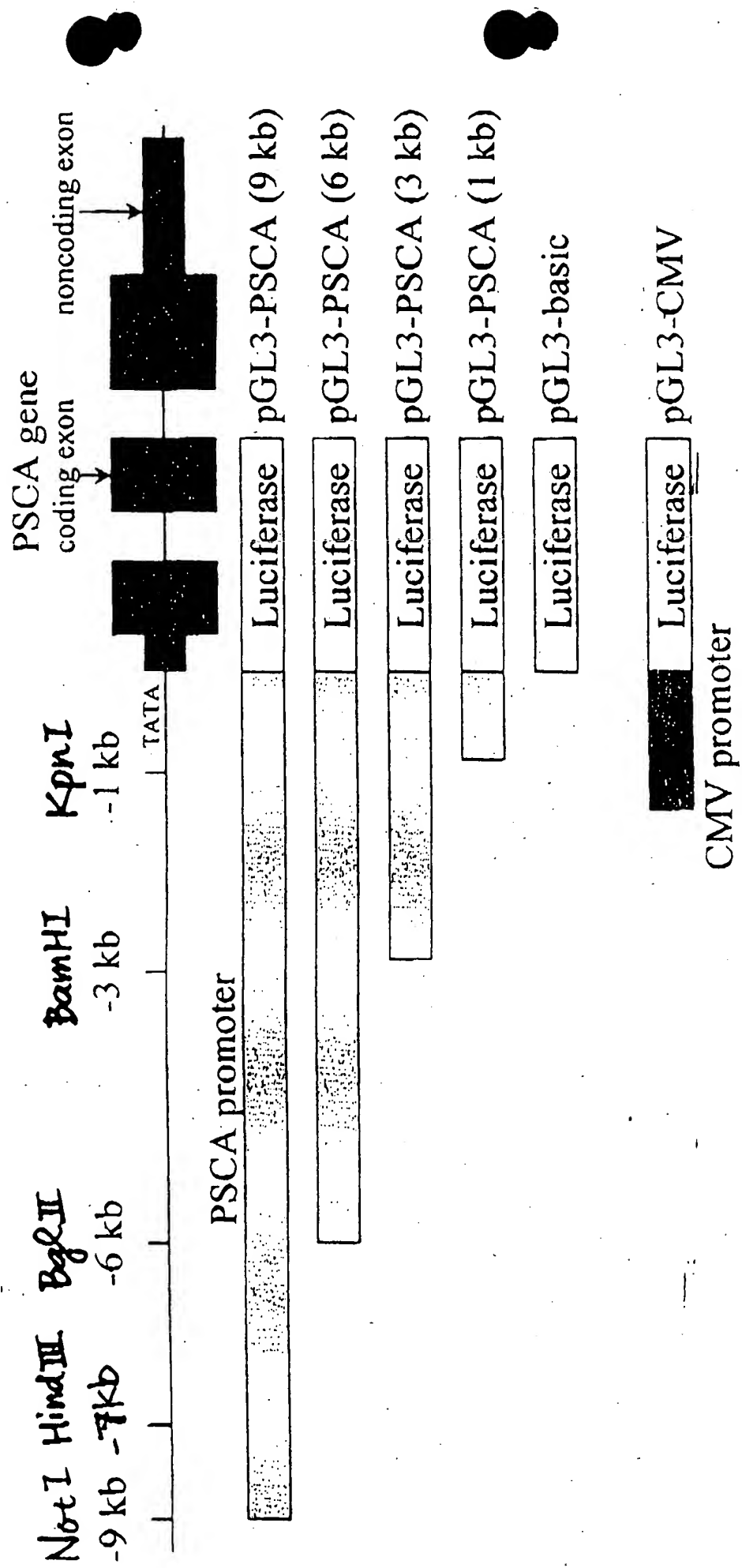


FIGURE 42

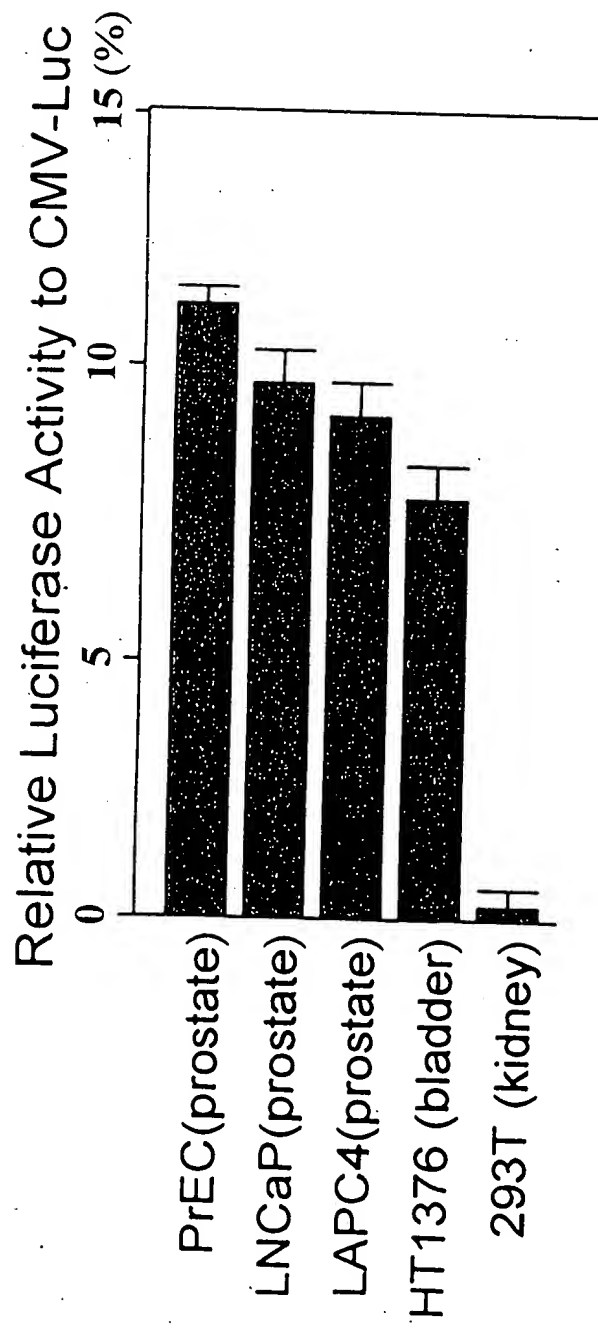


FIGURE 43

Identification of Prostate-Specific Elements Within PSCA Promoter Sequences

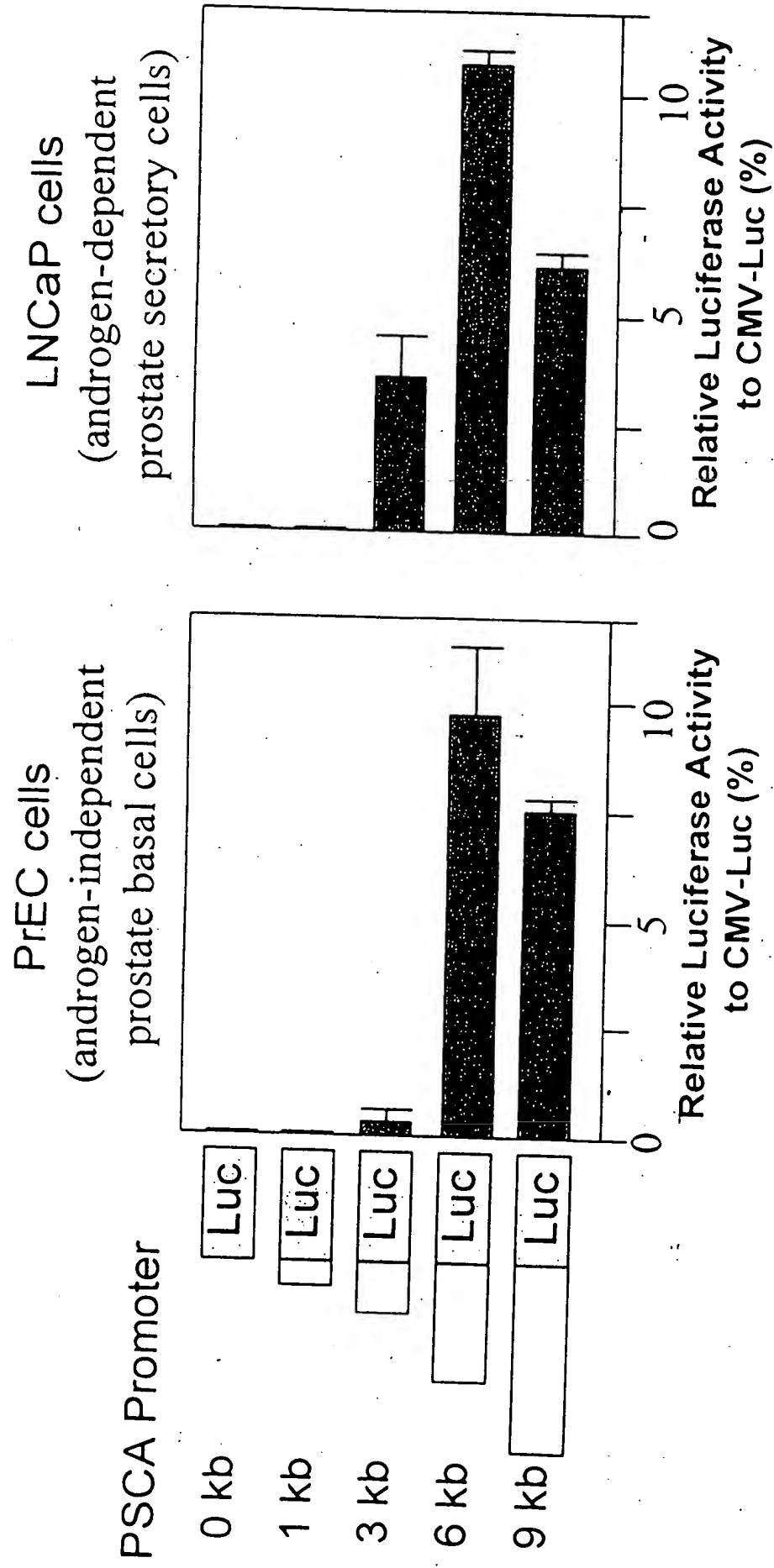


FIGURE 44

Update of Transgenic Mouse Projects

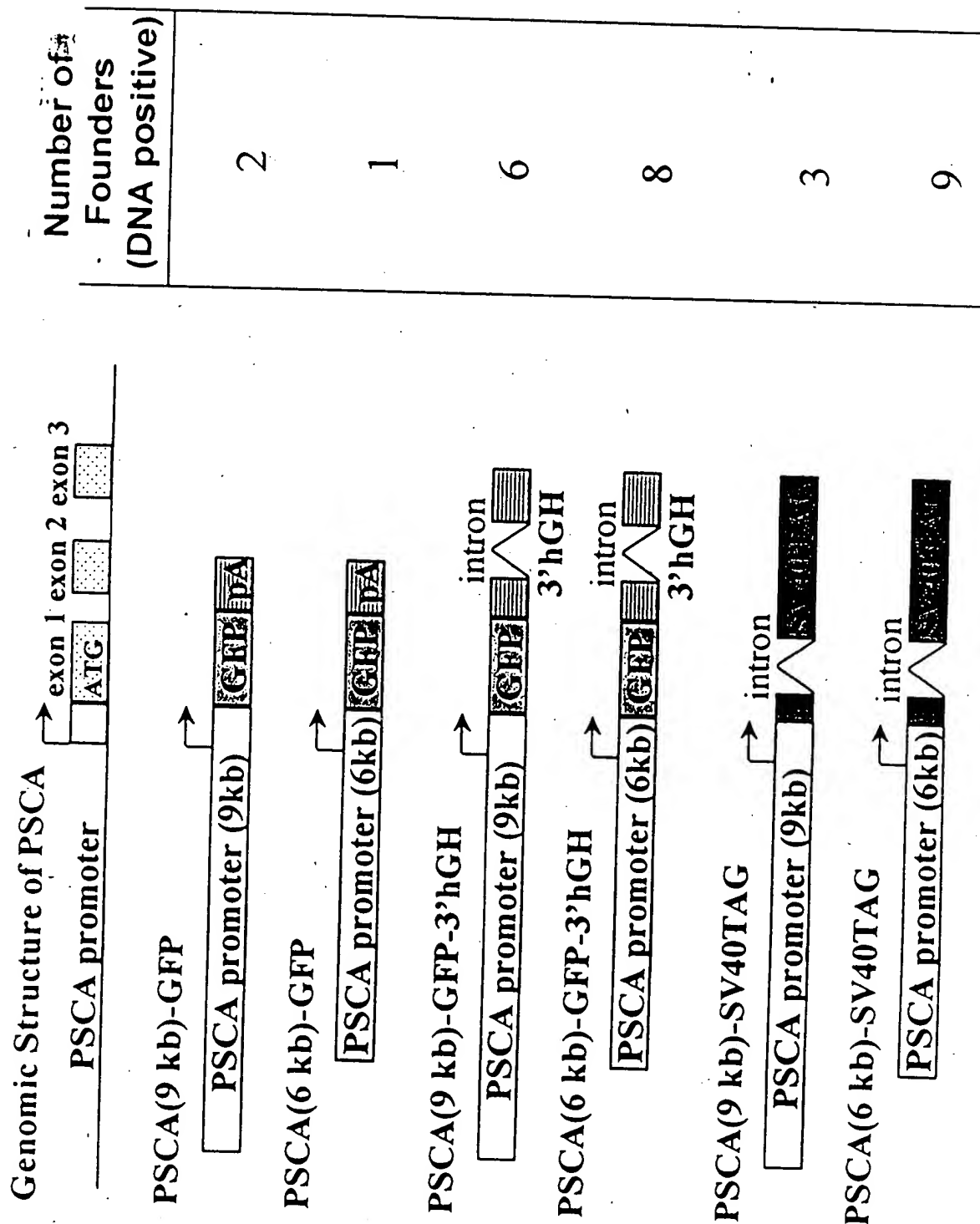


FIGURE 45

HUMAN
Spleen
Thymus
Prostate
Testis
Ovary
S. int.
Colon
PBL

Spleen
Thymus
Prostate
Testis

Thymus
Pro

Prostate
Test

estis
Ovary

any S.int.

Int.
Colon
HUMI

MAN
PBL

3

Heart

Brain

placenta

nta
ung
v!

iver

Muscle

anybody

anc

hPSCA→

Northern Analysis

MOUSE

Ant. prostate
Dorsol/at. prostate
Ventral prostate
Seminal vesicle
Urethra
Testis
Kidney

Sadder prostate
Urethral
Seminal

State
Central
Bladder pro.
Serratus

Seminal vesicle
Urethra
Prostate

Testis
Thra vesicle
Kidney

MOI

HOUSE

Stomach
of stomach
Duoden
Sm

Small intestine
Colon
Stomach
Duodenum

Salivary gland

thyroid gland









one marrow
skeletal
marrow

arrow
part
rain.
atal muscle

ing
e
scale

ing
ver
rin

in

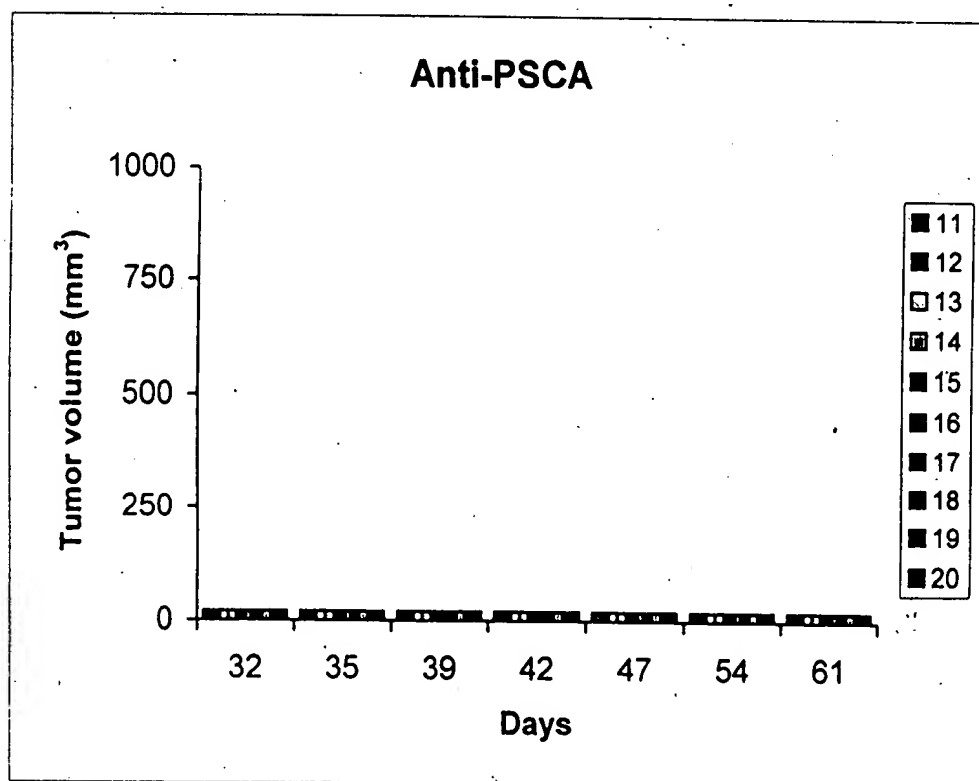
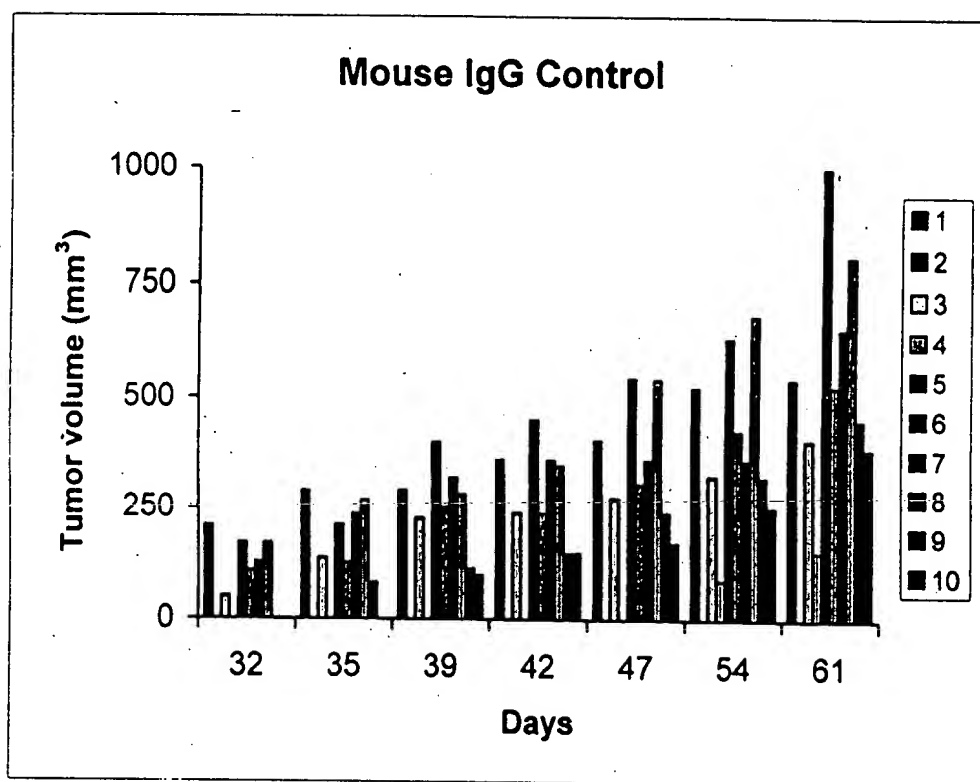


mPSCA →

mG3PDH \uparrow

RT-PCR

FIGURE 47



A

FIG. 49

Epitope recognized (OD 450 nm)

<u>mAb</u>	<u>Isotype</u>	<u>F (18-98)</u>	<u>N (2-50)</u>	<u>M (46-109)</u>	<u>C (85-123)</u>
1G8	IgG1 k	1.485	0.004	1.273	0.003
2A2	IgG2a k	0.973	0.631	0.023	0.010
2H9	IgG1 k	1.069	1.026	0.002	0.001
3C5	IgG2a k	1.916	1.709	0.006	0.002
3E6	IgG3 k	1.609	0.036	1.133	2.118
3G3	IgG2a k	2.805	1.731	0.004	0.000
4A10	IgG2a k	1.053	0.493	0.000	0.001

B

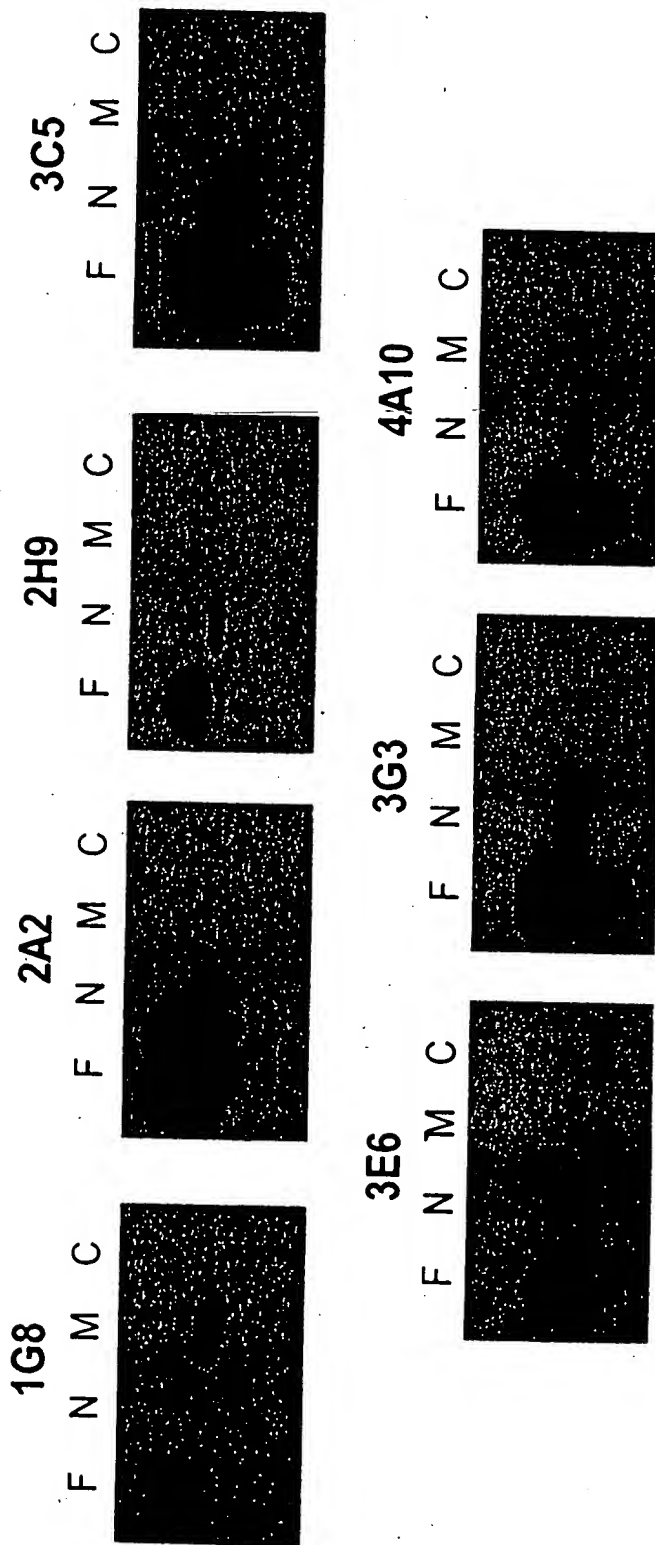
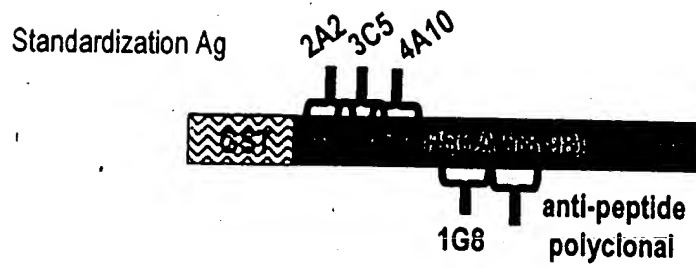
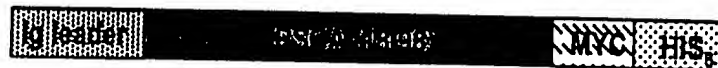


FIG. 50

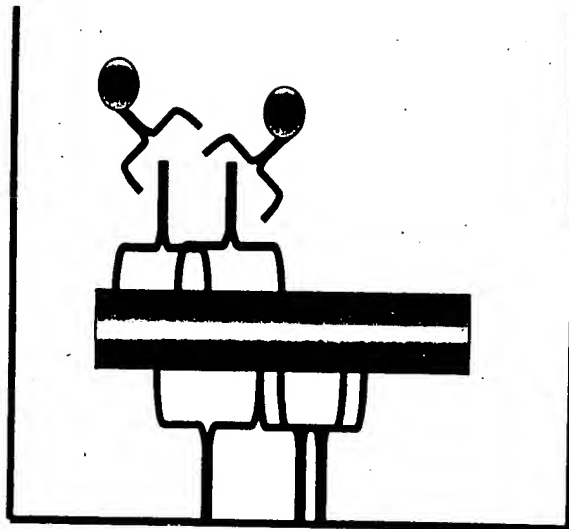
A



Engineered mammalian secreted form



B



Anti-IgG2a HRP

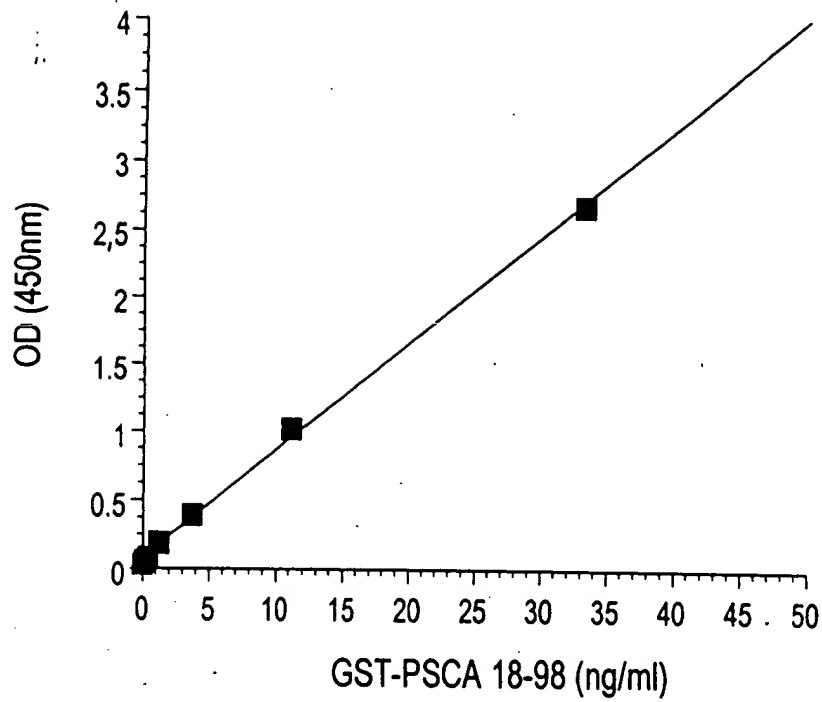
Anti-PSCA mAbs 3C5+4A10+2A2 (IgG2a)

PSCA

Affinity purified anti-peptide polyclonal
+ mAb 1G8 (IgG1)

FIG. 51

A



B

<u>Sample</u>	<u>OD+range (n=2)</u>	<u>ng/ml</u>
vector	0.005+0.001	ND
vector+hu serum	0.004+0.001	ND
secPSCA	2.695+0.031	32.92
secPSCA+hu serum	2.187+0.029	26.55

FIG. 52

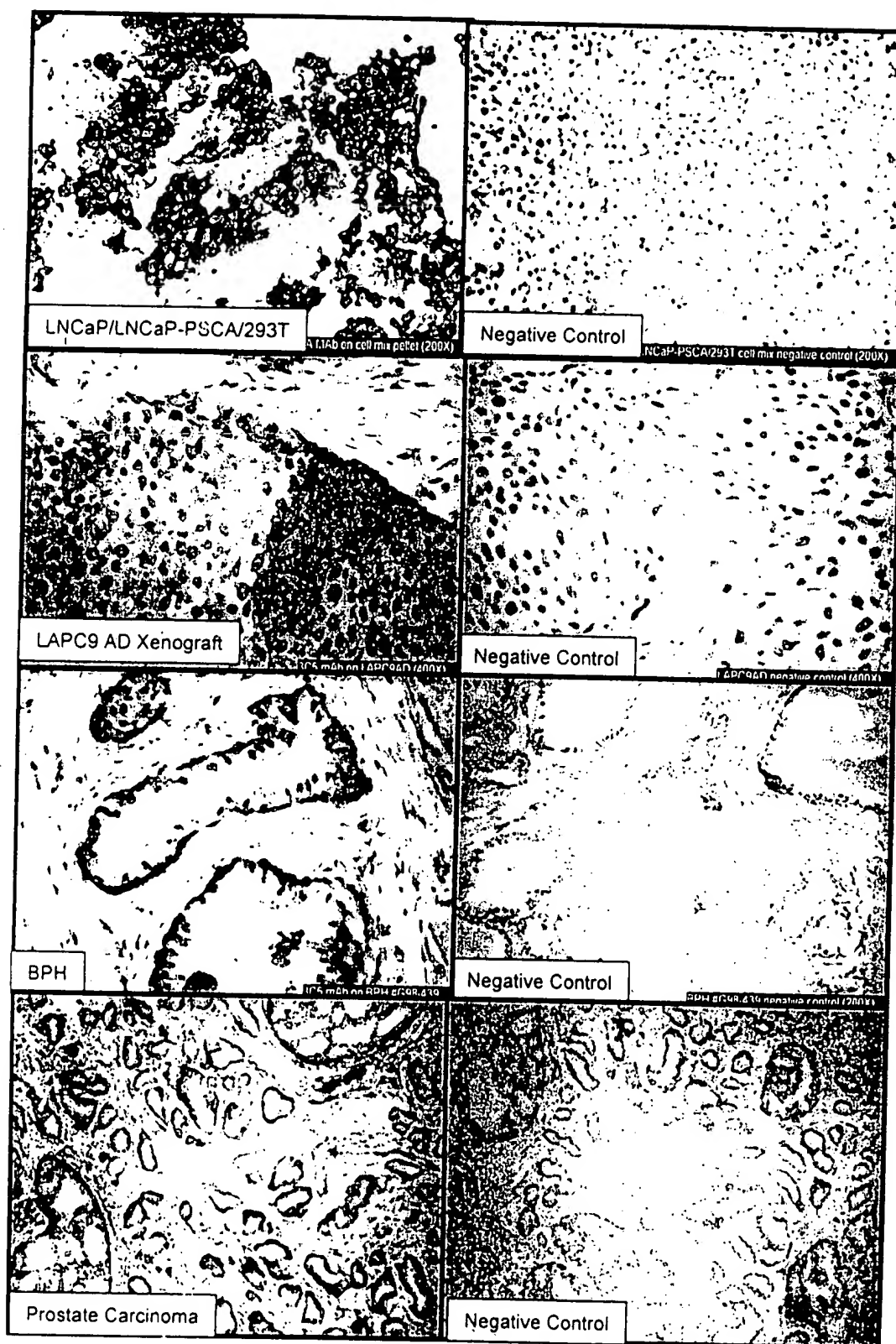
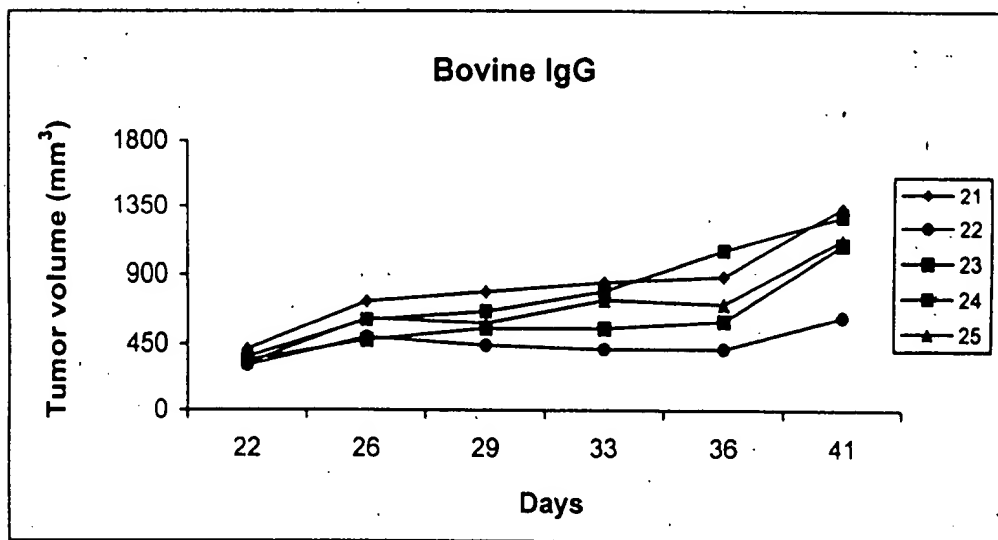
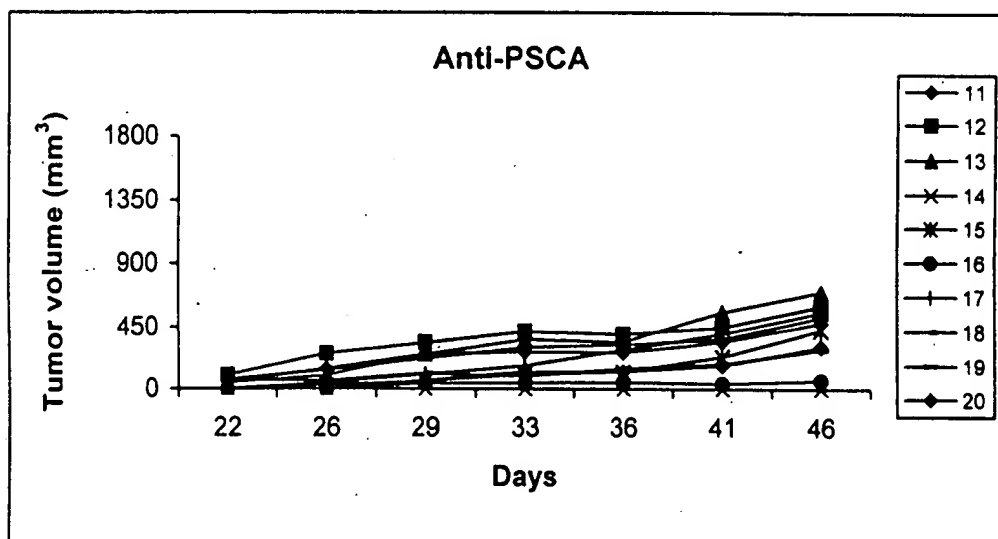
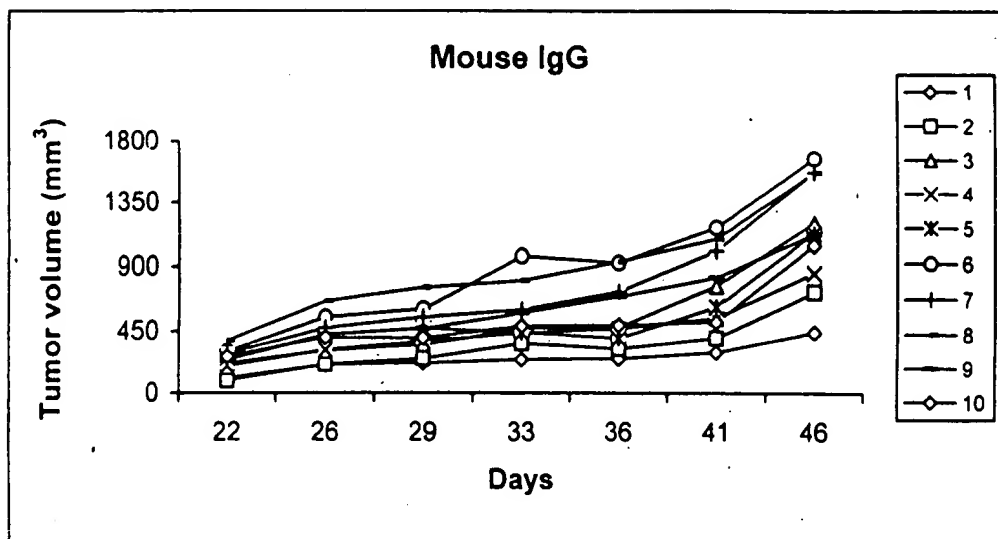


FIG. 53



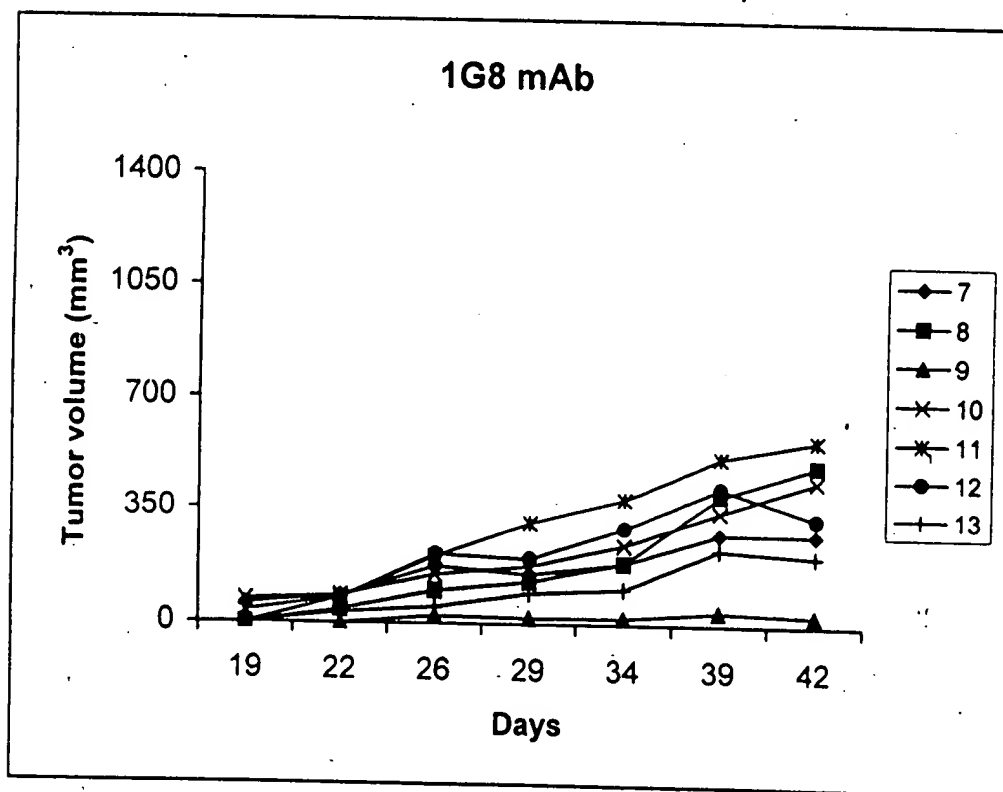
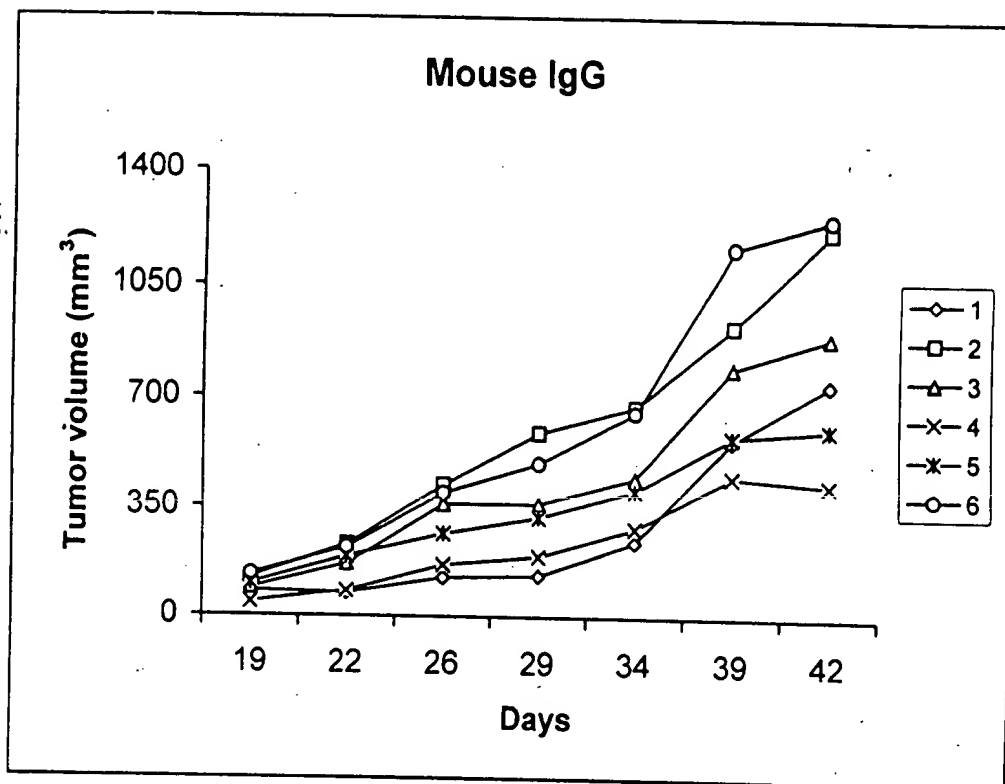
[illegible]

FIG. 55

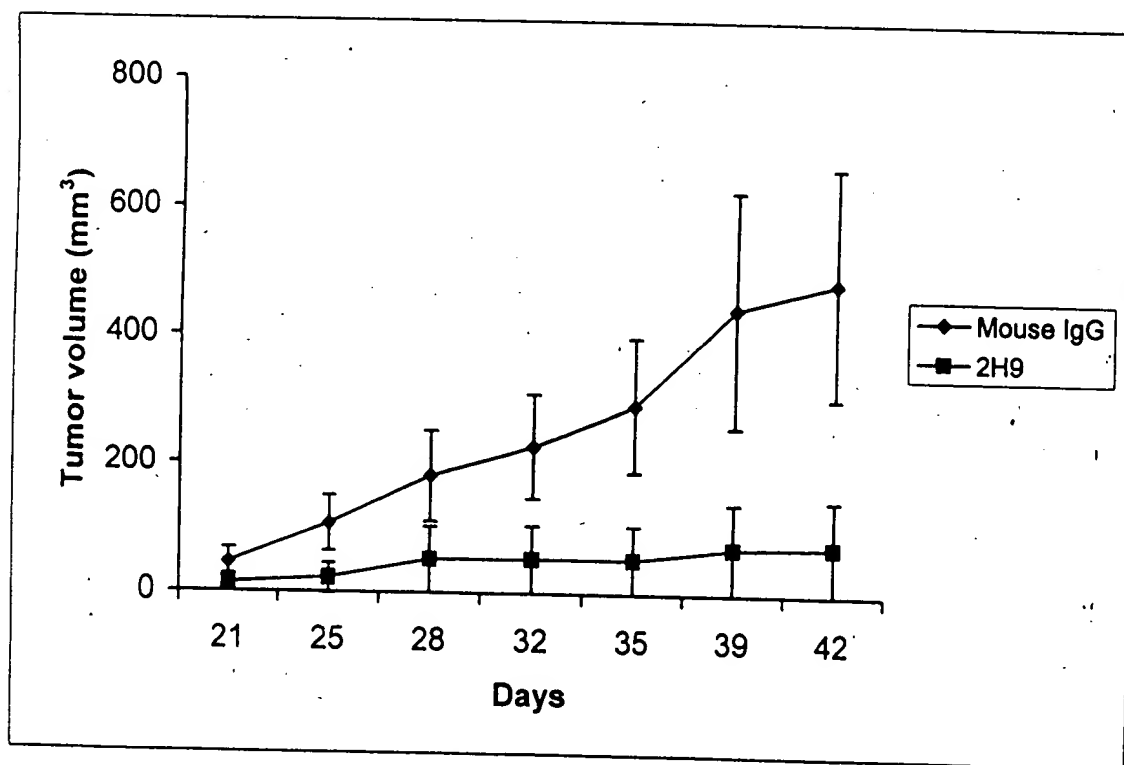
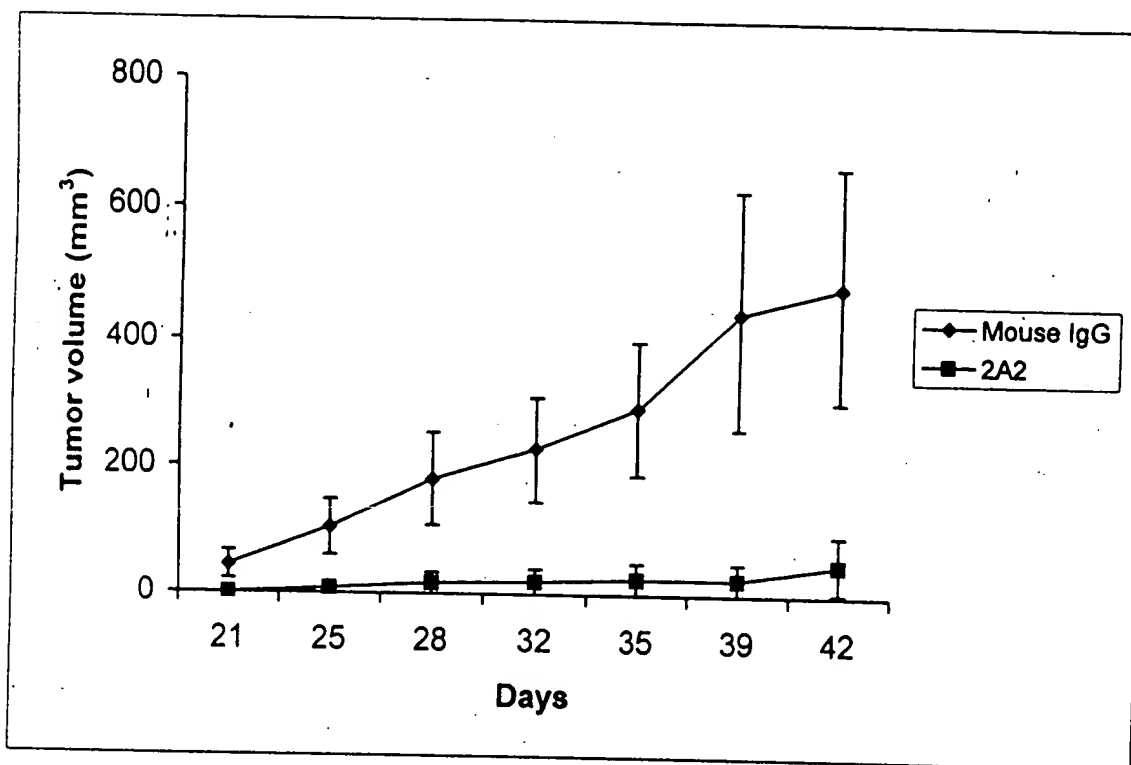


FIG. 56

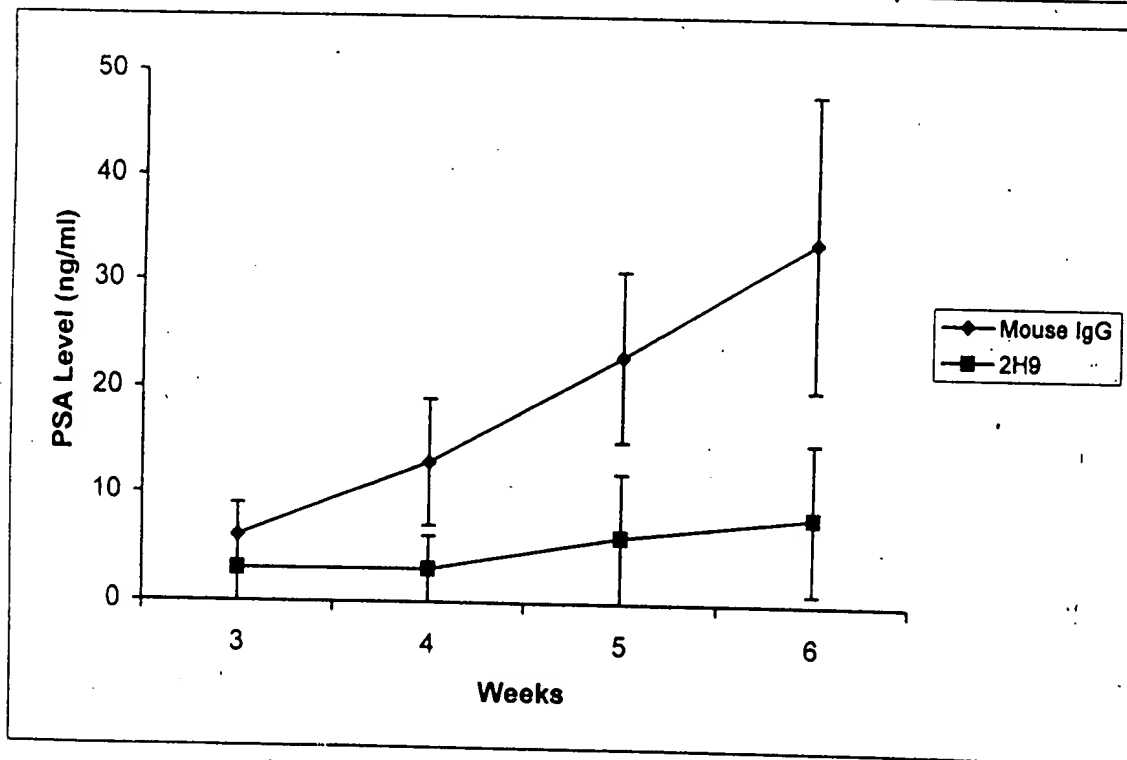
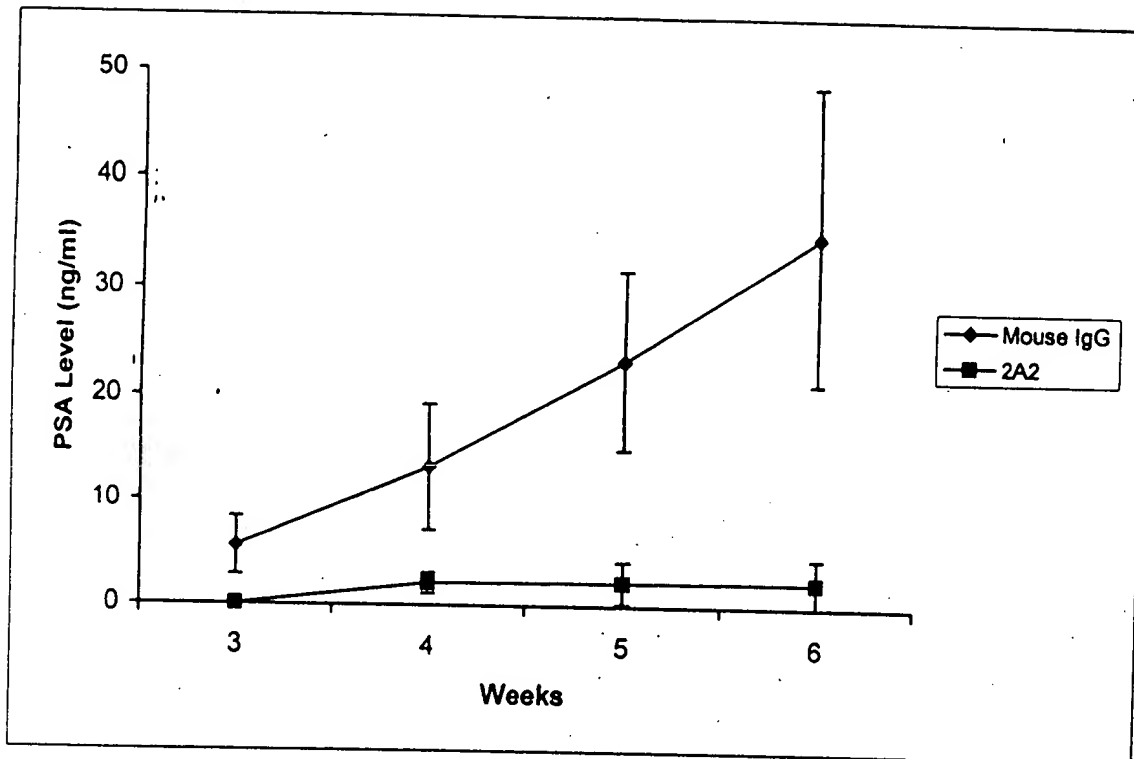
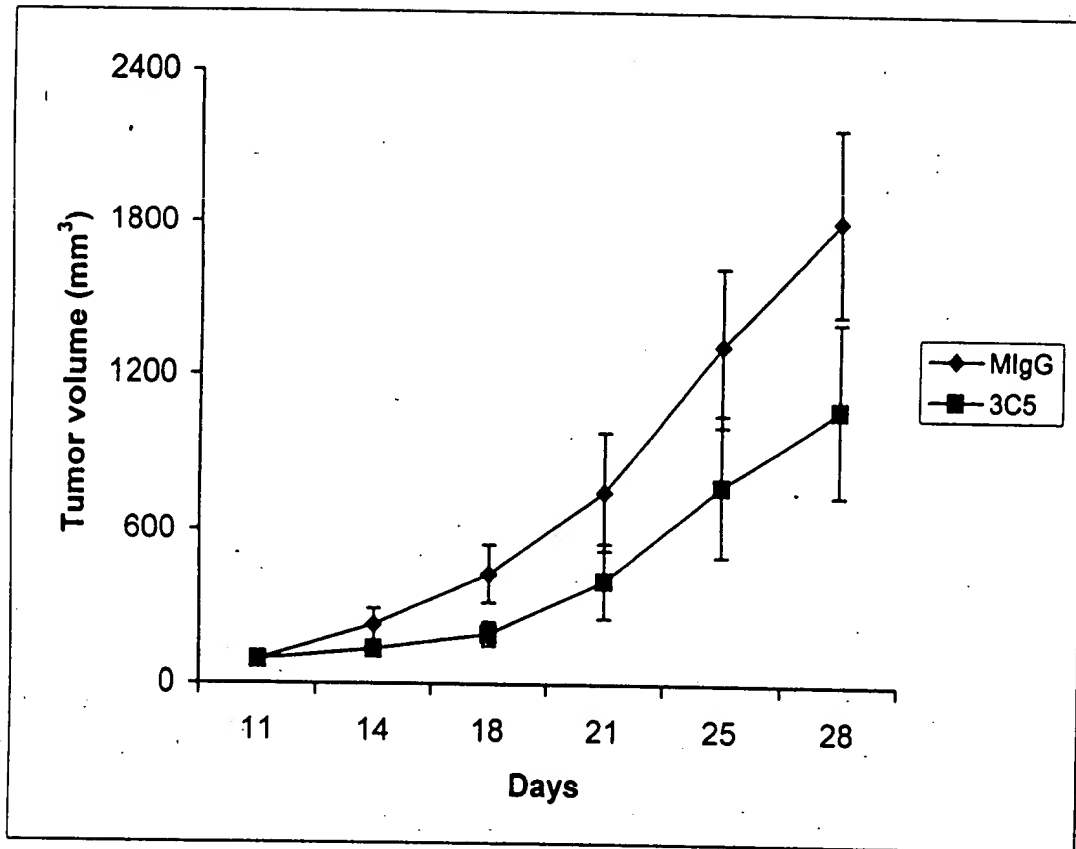


FIG. 57





Lips

GGGGCAGAACTTGTGAGGTCAGGGGCCTCAGTCAAGTTGTCCTGCACAGCTTCTGGCTTC 120
G A E L V R S G A S V K L S C T A S G F 40

CDR2
 ATTGGATGGATTGATCCTGAGAATGGTGACACTGAATTTGTCCCGAAGTTCCAGGGCAAG 240
 I G W I D P E N G D T E F V P K F O G K 80

GCCACTATGACTGCAGACATTTTCTCCAACACAGCCTACCTGCACCTCAGCAGCCTGACA 300
A T M T A D I F S N T A Y L H L S S L T 100

TCTGAAGACACTGCCGTCTATTACTGTAAACGGGGGGTTTCTGGGGCCAAGGGACTCTG 360
S E D T A V Y Y C K T G G F W G Q G T L 120

GTCACTGTCTCTGCAGCCAAAACGACACCCCCATCTGTCTATCCACTG
V T V S A A K T T P P S V Y P L

FIG. 59

TTGGTAGCAACAGCCTCAGATGTCCACTCCCAGGTCCAAGTGCAGCAACCTGGGTCTGAA 60
L V A T A S D V H S Q V Q L Q Q P G S E 20

CTGGTGAGGCCTGGAAGCTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTATACATTCTCC 120
L V R P G T S V K L S C K A S G Y T F S 40
CDR1

AGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAAAT 180
S Y W M H W V K Q R P G Q G L E W I G N 60

ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240
I D P G S G Y T N Y A E N L K T K A T L 80
CDR2

ACTGTAGACACATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGAC 300
T V D T S S S T A Y M Q L S S L T S E D 100

TCTGCAGTCTATTACTGTACAAGCCGATCTACTATGATTACGACGGGATTGCTTACTGG 360
S A V Y Y C T S R S T M I T T G F A Y W 120
CDR3

GGCCAAGGGACTCTGGTCACTGTCTCTGCAGCTACAACAACAGCCCCATCTGTCTATCCA 420
G Q G T L V T V S A A T T T A P S V Y P 160

CTGGCC
L A

FIG. 60

AATGACTTCGGGTTGAGCTGGGTTTTTATTATTGTTCTTTTAAAGGGGTCCGGAGTGAA 60
N D F G L S W V F I I V L L K G V R S E 20

GTGAGGCTTGAGGAGTCTGGAGGAGGCTGGGTGCAACCTGGAGGATCCATGAAACTCTCC 120
V R L E E S G G G W V Q P G G S M K L S 40

TGTGTAGCCTCTGGATTTACTTTTCAGTAATTACTGGATGACTTGGGTCCGCCAGTCTCCA 180
C V A S G F T F S N Y W M T W V R Q S P 60
CDR1

GAGAAGGGGCTTGAGTGGGTTGCTGAAATTCGATTGAGATCTGAAAATTATGCAACACAT 240
E K G L E W V A E I R L R S E N Y A T H 80
CDR2

TATGCGGAGTCTGTGAAAGGGAAATTCACCATCTCAAGAGATGATTCCAGAAGTCGTCTC 300
Y A E S V K G K F T I S R D D S R S R L 100

TACCTGCAAATGAACAACTTAAGACCTGAAGACAGTGGGAATTTATTACTGTACAGATGGT 360
Y L Q M N N L R P E D S G I Y Y C T D G 120

CTGGGACGACCTAACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCCAAAACGACA 420
L G R P N W G Q G T L V T V S A A K T T 140
CDR3

CCCCATCTGTCTATCCACTGGCCCCTTGTA
P P S V Y P L A P C V

Sequence

FIG. 61

CDR1 Comparisons

1G8	1gG _{1k}	Middle	G	F	N	I	K	D	Y	Y	I	H
2H9	1gG _{1k}	N-Term.	G	F	T	F	S	N	Y	W	M	T
4A10	1gG _{2ak}	N-Term.	G	Y	T	F	S	S	Y	W	M	H

CDR2 Comparisons

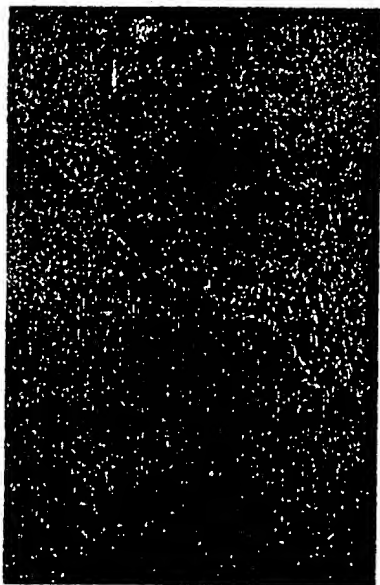
1G8	1gG _{1k}	W	I	D	P	E	N	G	D	T	E	F	V	P	K	F	Q	G		
2H9	1gG _{1k}	E	I	R	L	R	S	E	N	Y	A	T	H	Y	A	E	S	V	K	G
4A10	1gG _{2ak}	N	I	D	P	G	S	G	Y	T	N	Y	A	E	N	L	K	T		

CDR3 Comparisons

1G8	1gG _{1k}	G	G	F													
2H9	1gG _{1k}	L	G	R	P	N											
4A10	1gG _{2ak}	R	S	T	M	I	T	T	G	F	A	Y					

FIG. 62

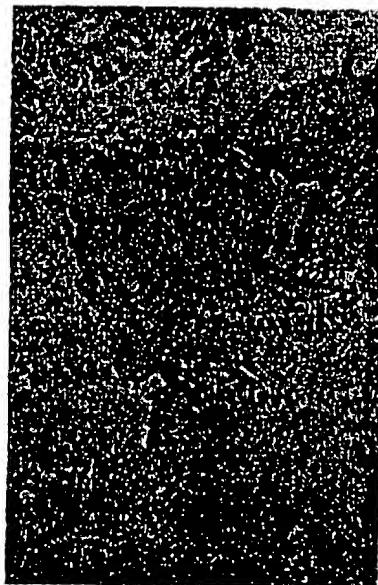
A



B



C



D

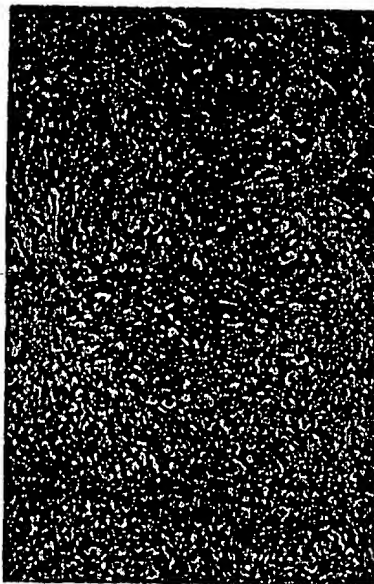


FIG. 63

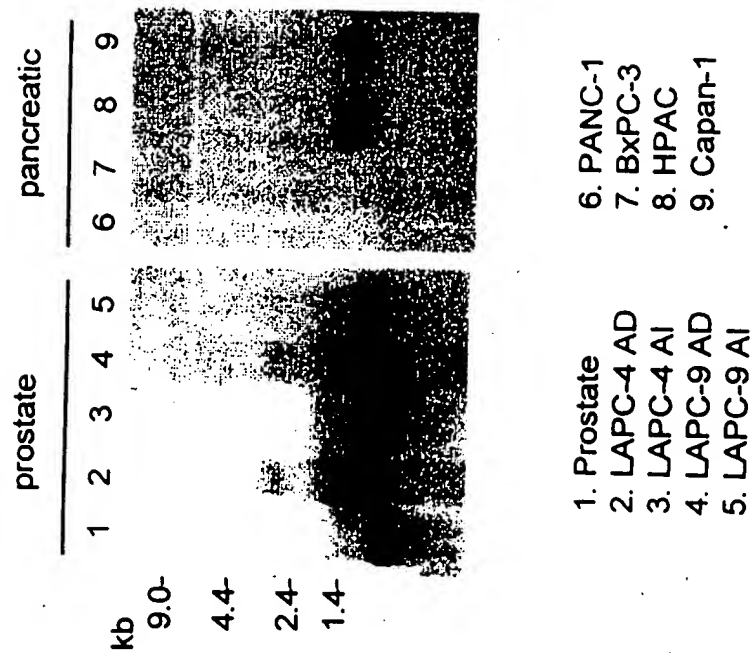
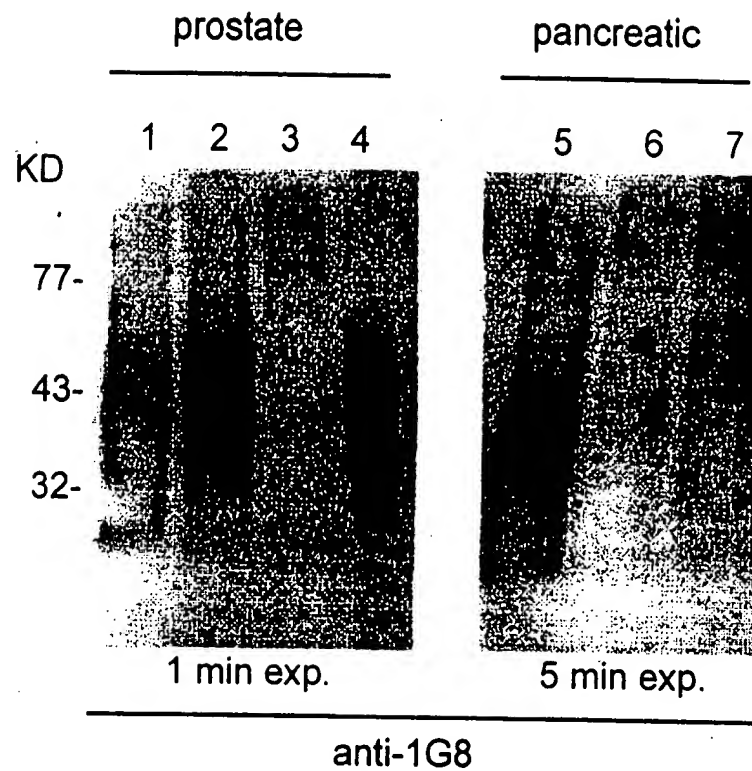


FIG. 64



1. LAPC-4 AD
2. LAPC-9 AI
3. LNCaP
4. LNCaP-PSCA
5. HPAC
6. Capan-1
7. ASPC-1

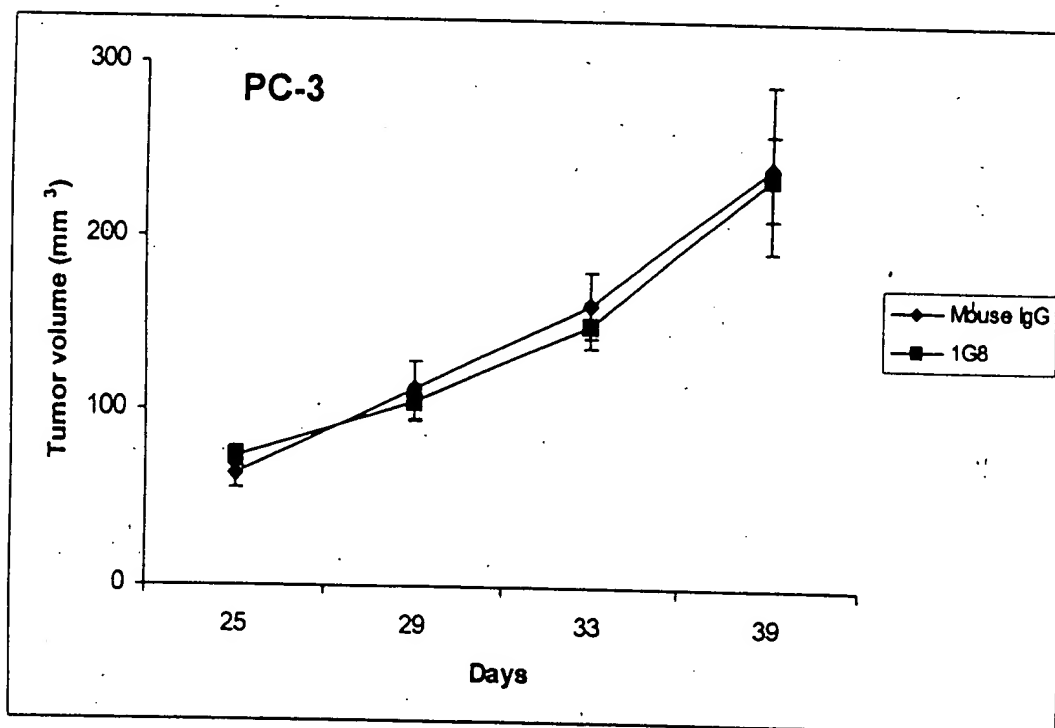
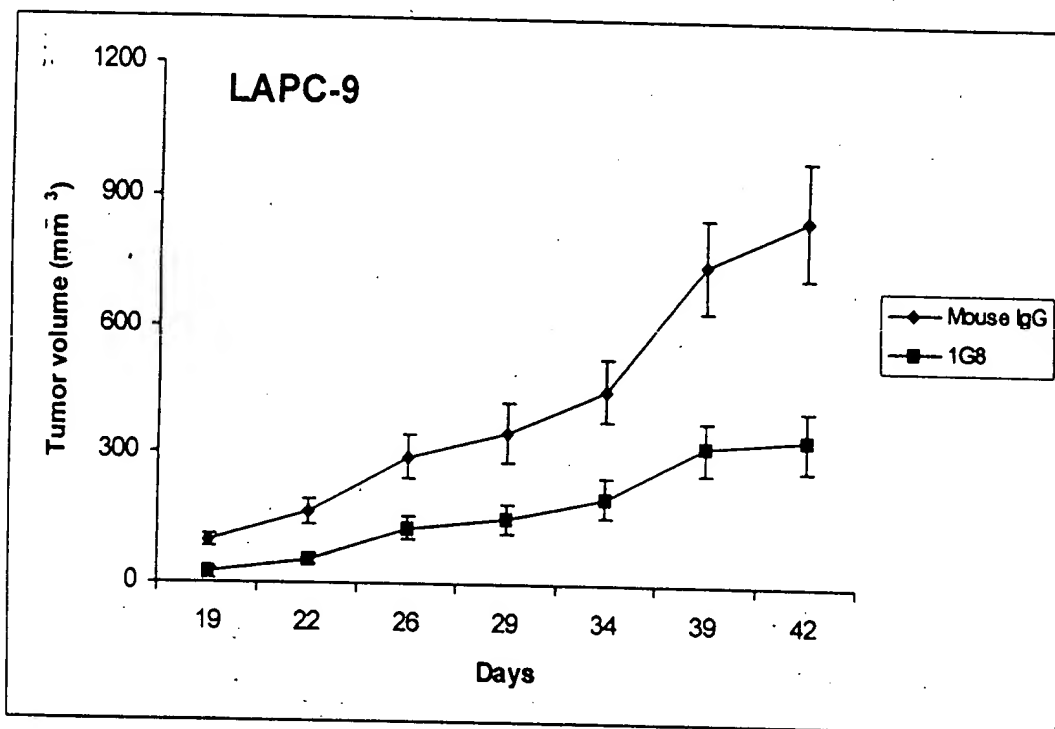
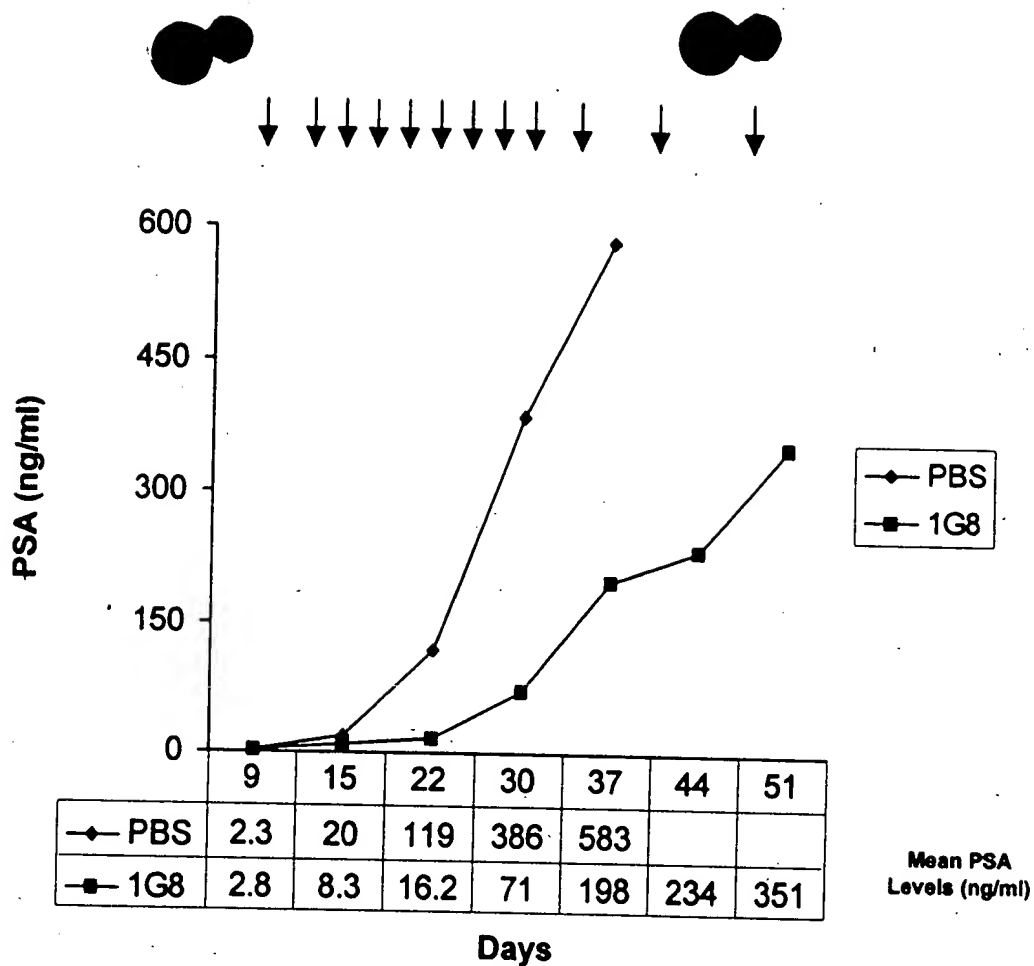


FIGURE 65

A)



B)

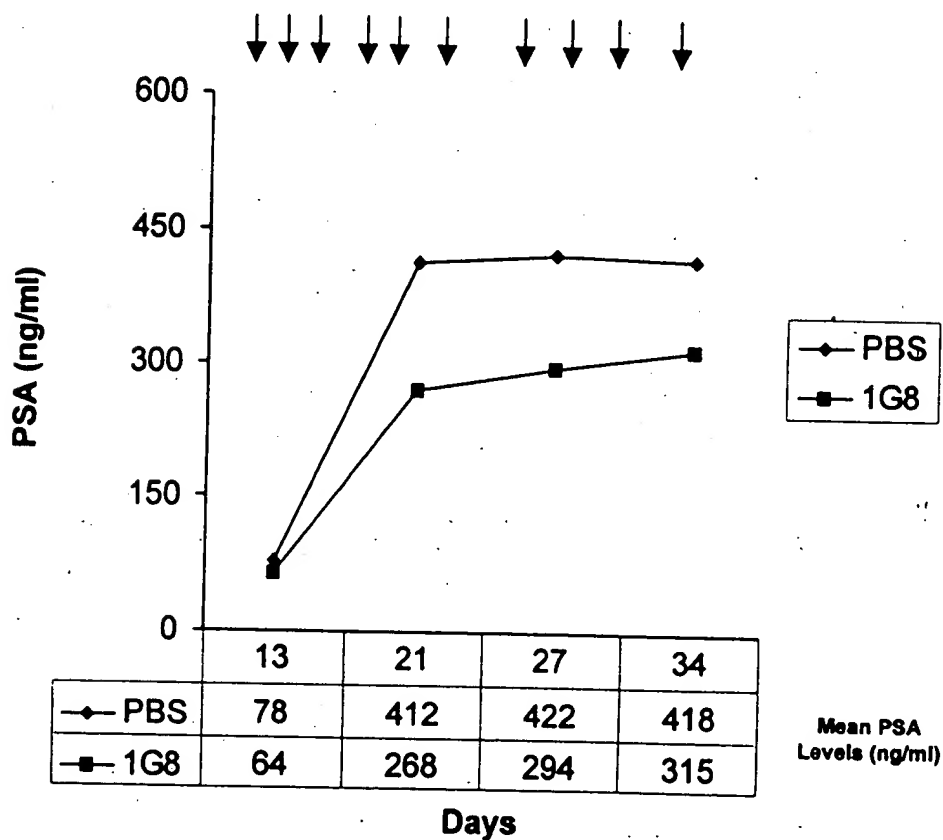
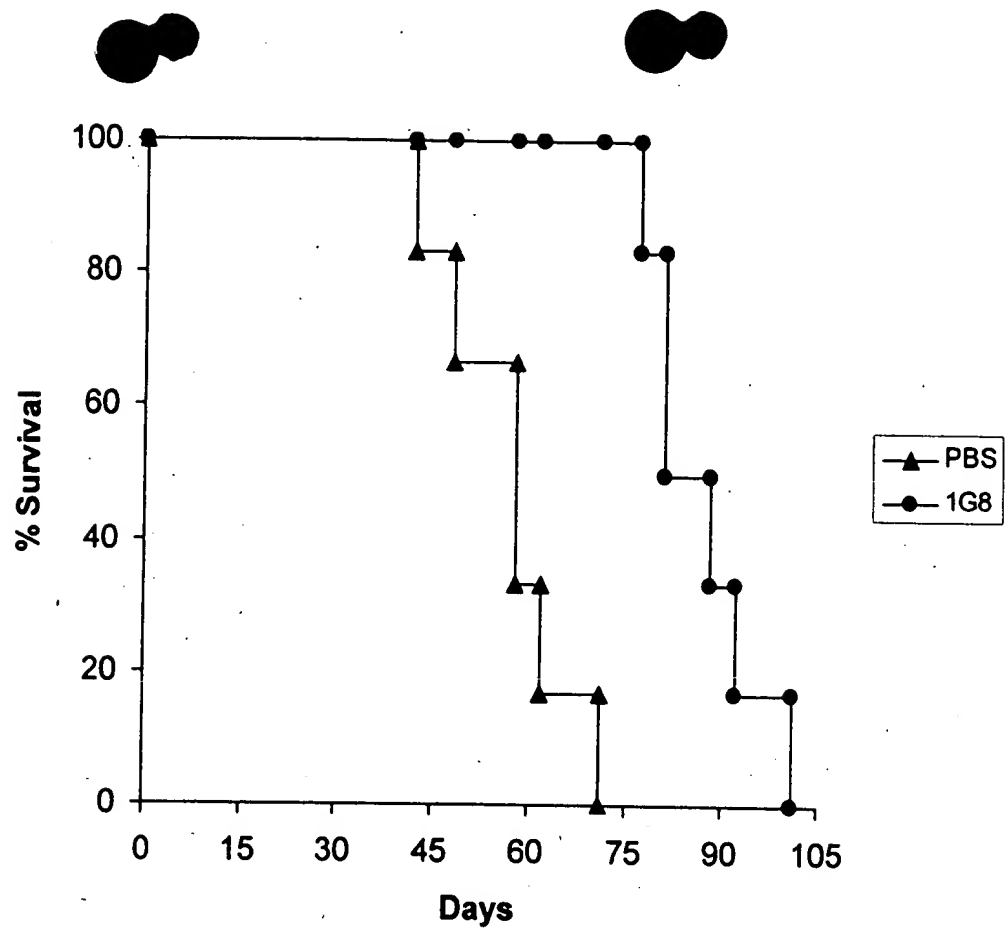


Figure 66

A)



B)

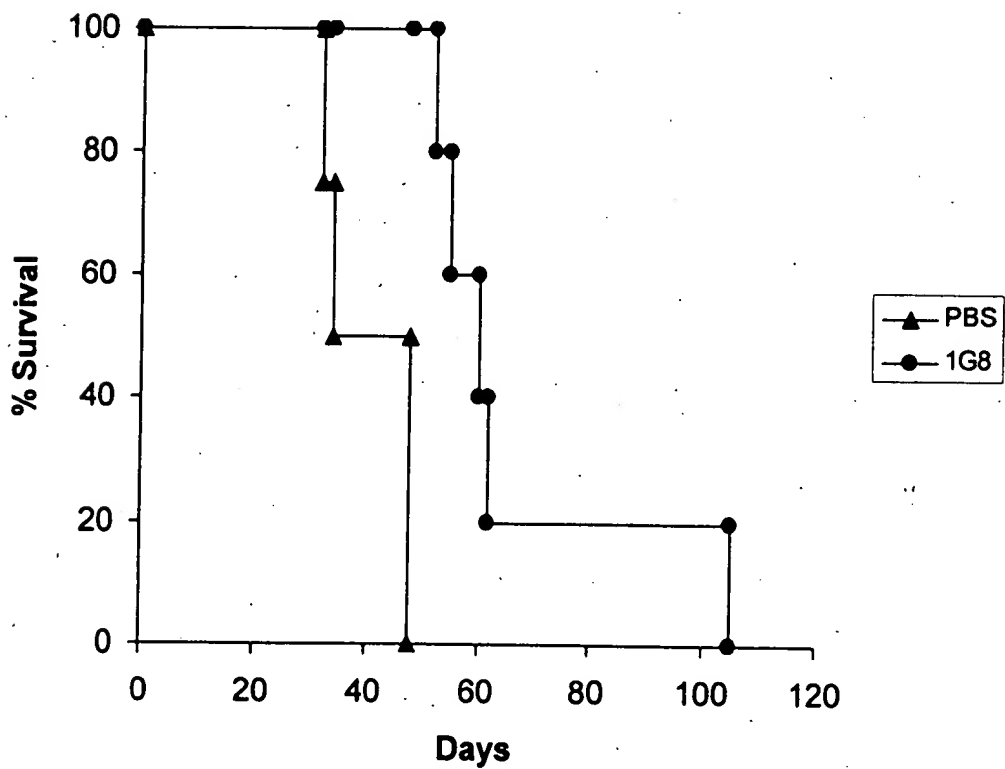
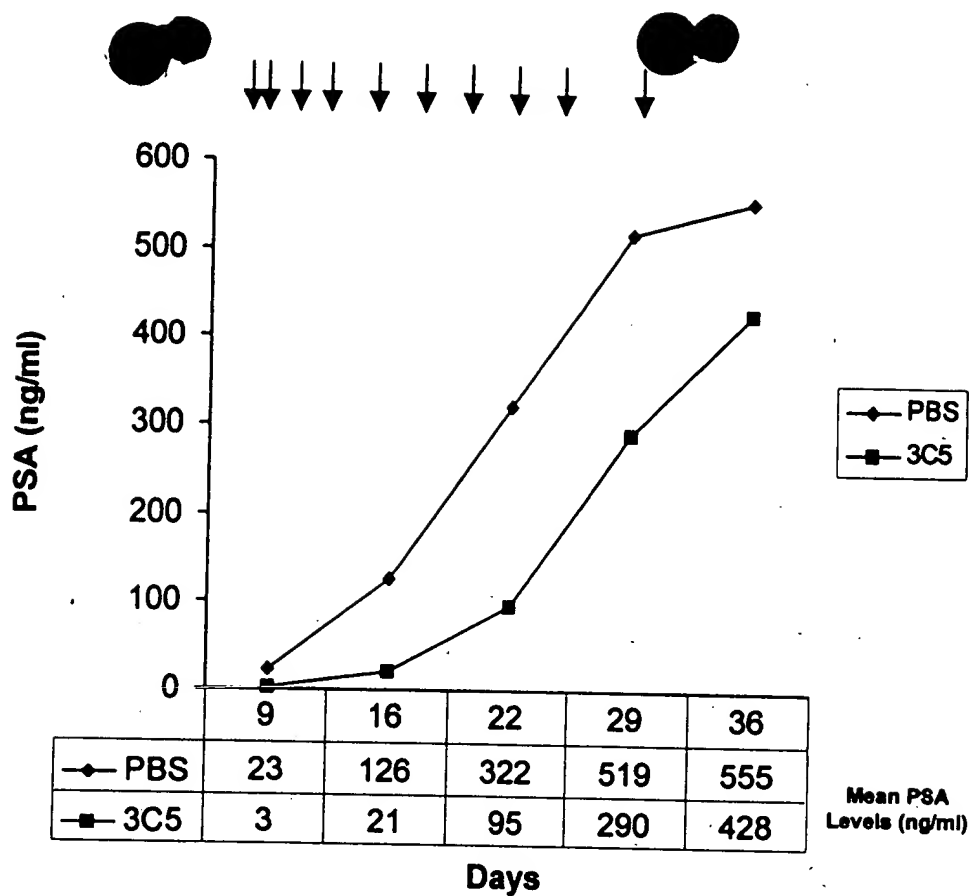


Figure 67

A)



B)

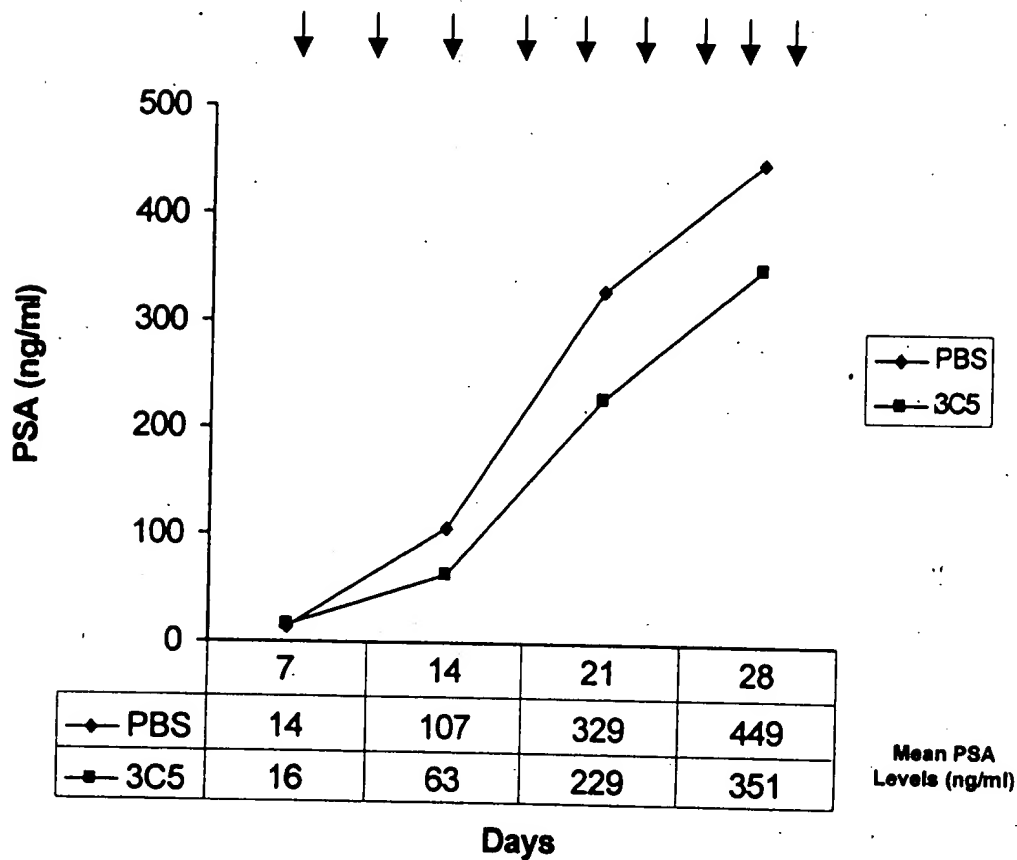
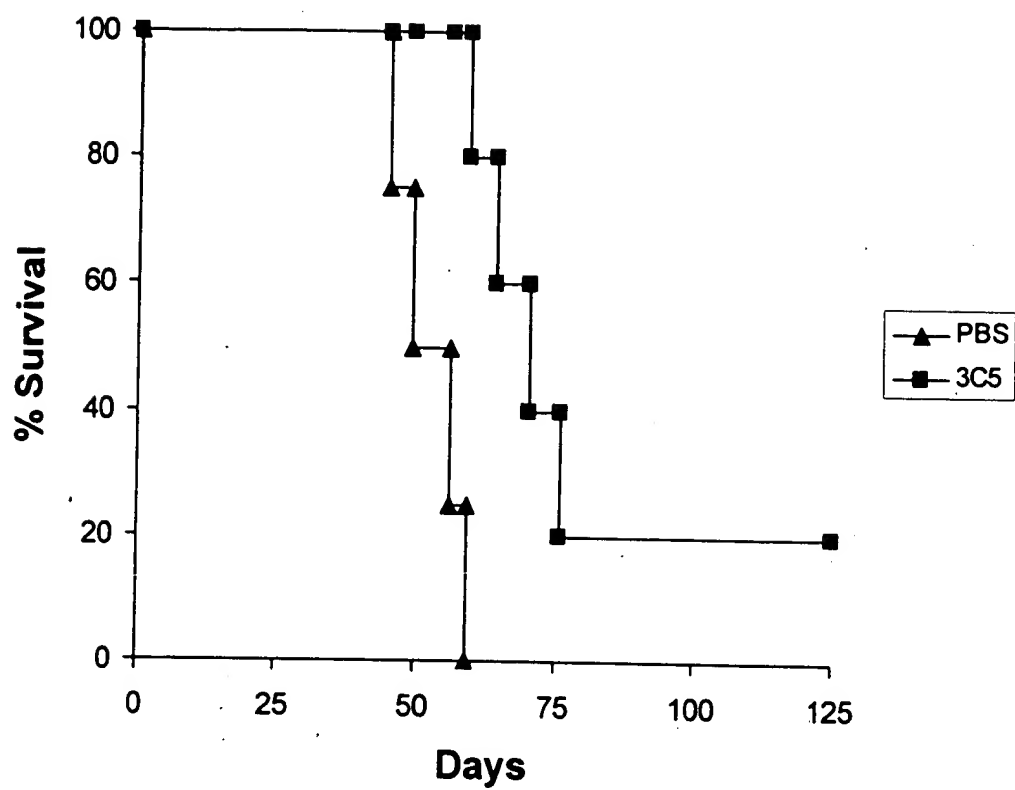


Figure 68

A)



B)

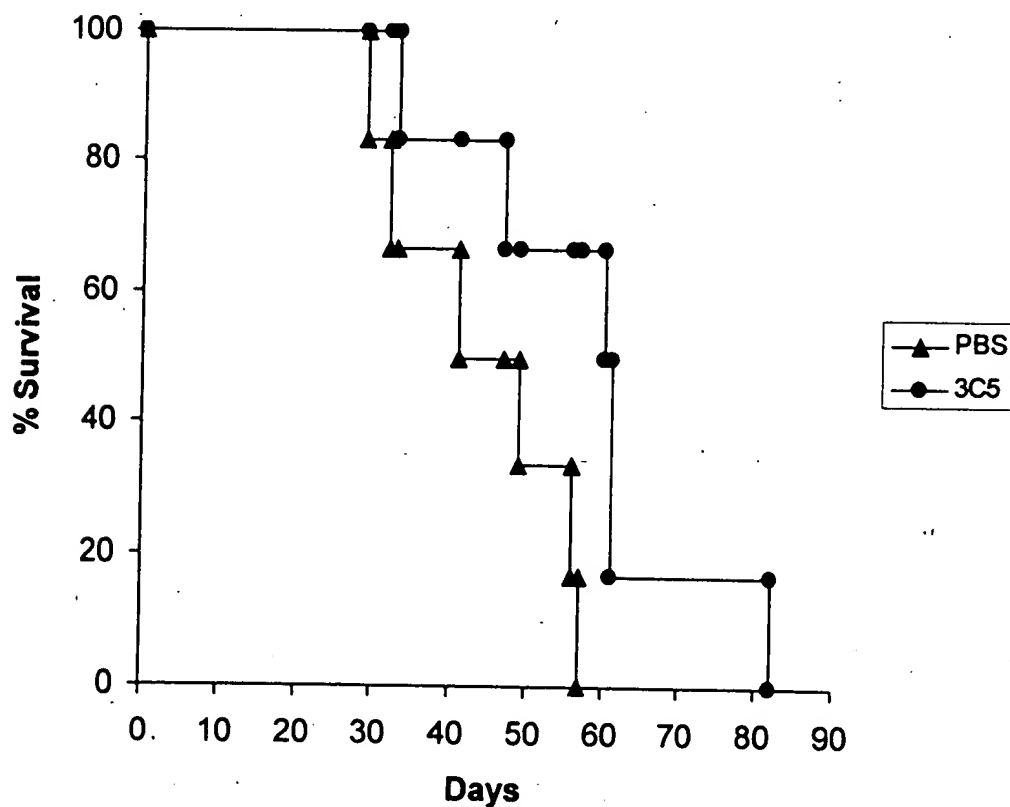


Figure 69

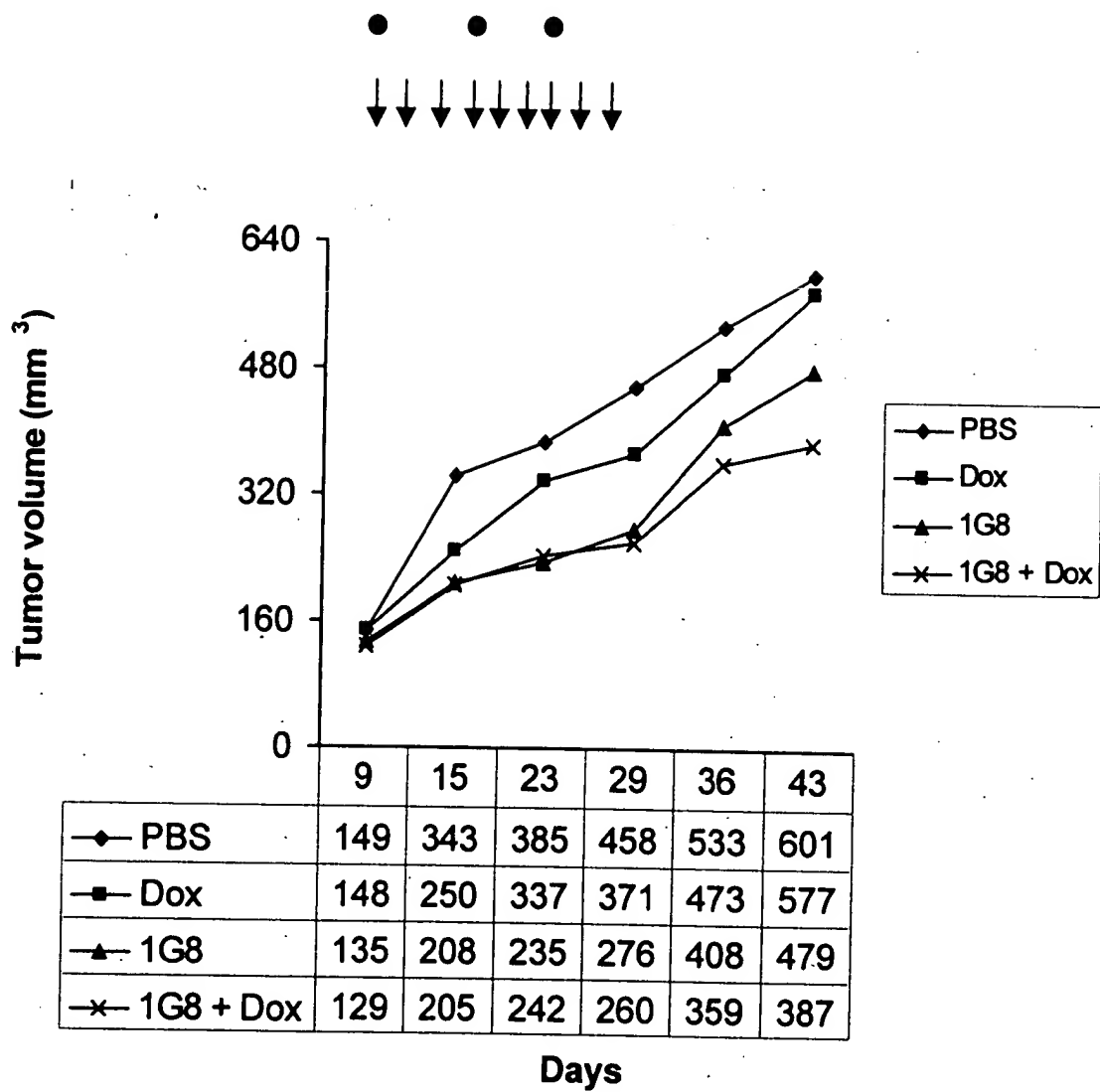
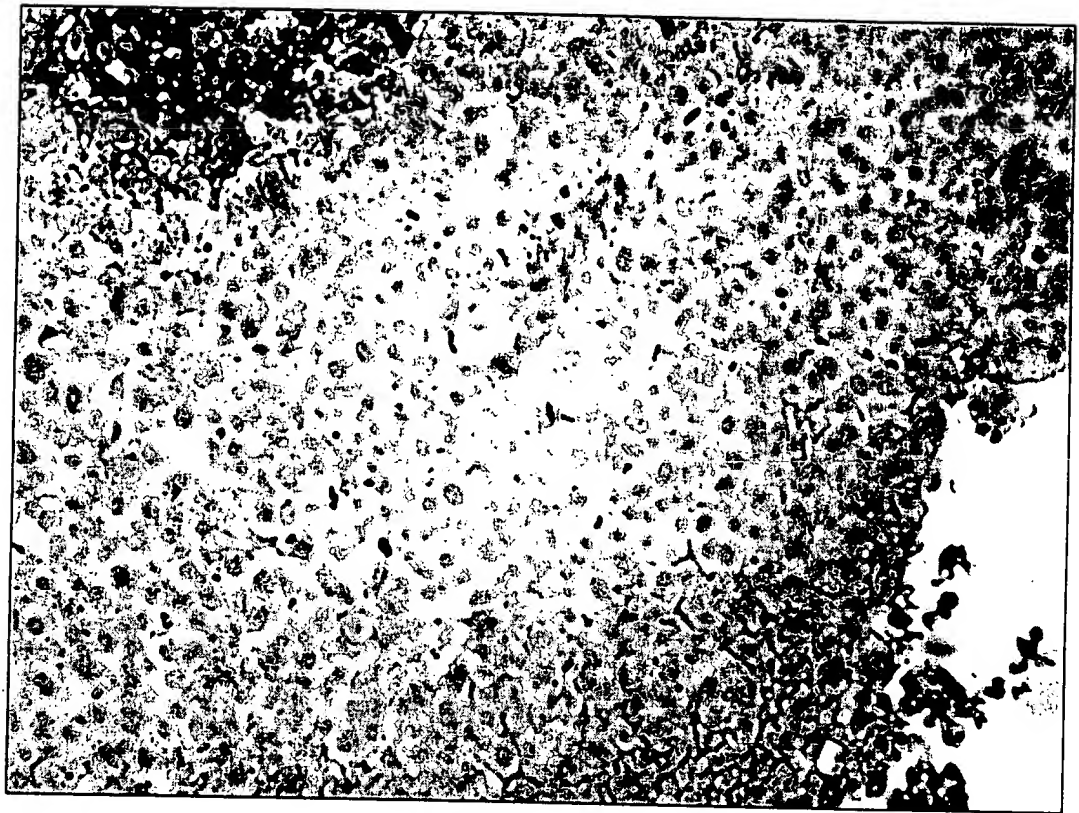


Figure 70

PSCA 3C5 MAb Localizes within LAPC9AD Xenograft Tissue

3C5 Treated



mIgG Treated

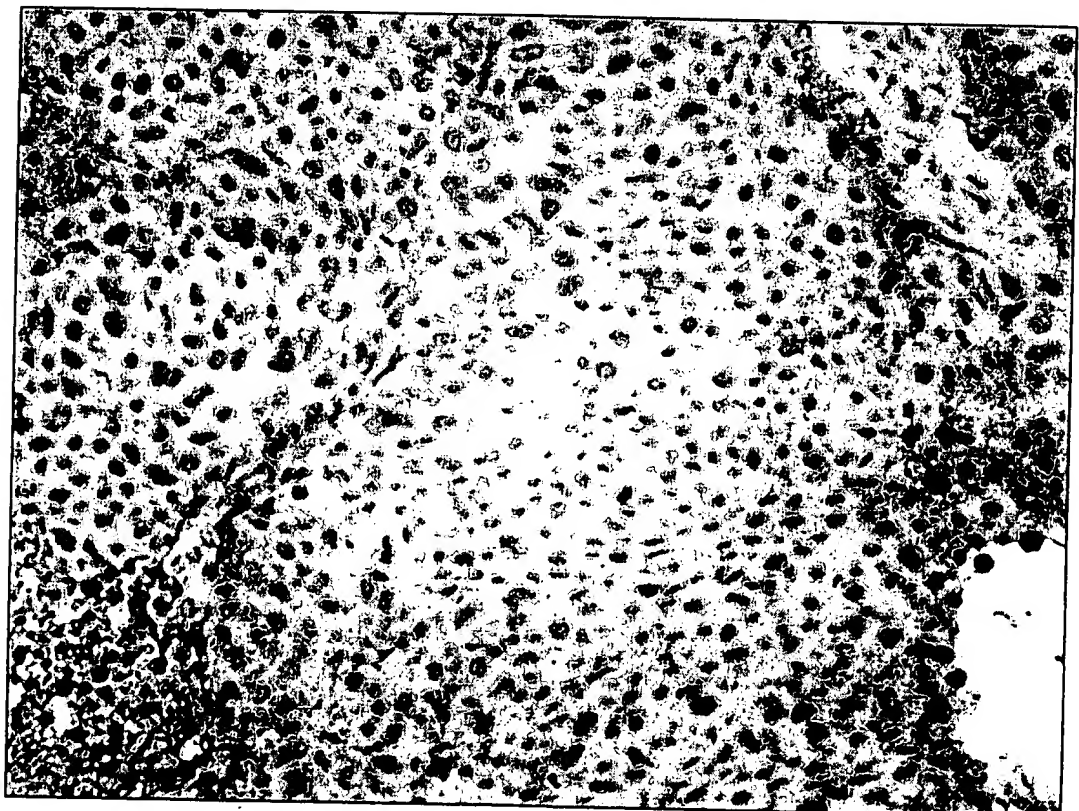


Figure 71

3C5 Anti-PSCA MAb is Localized to Established LAPC-9 Tumors

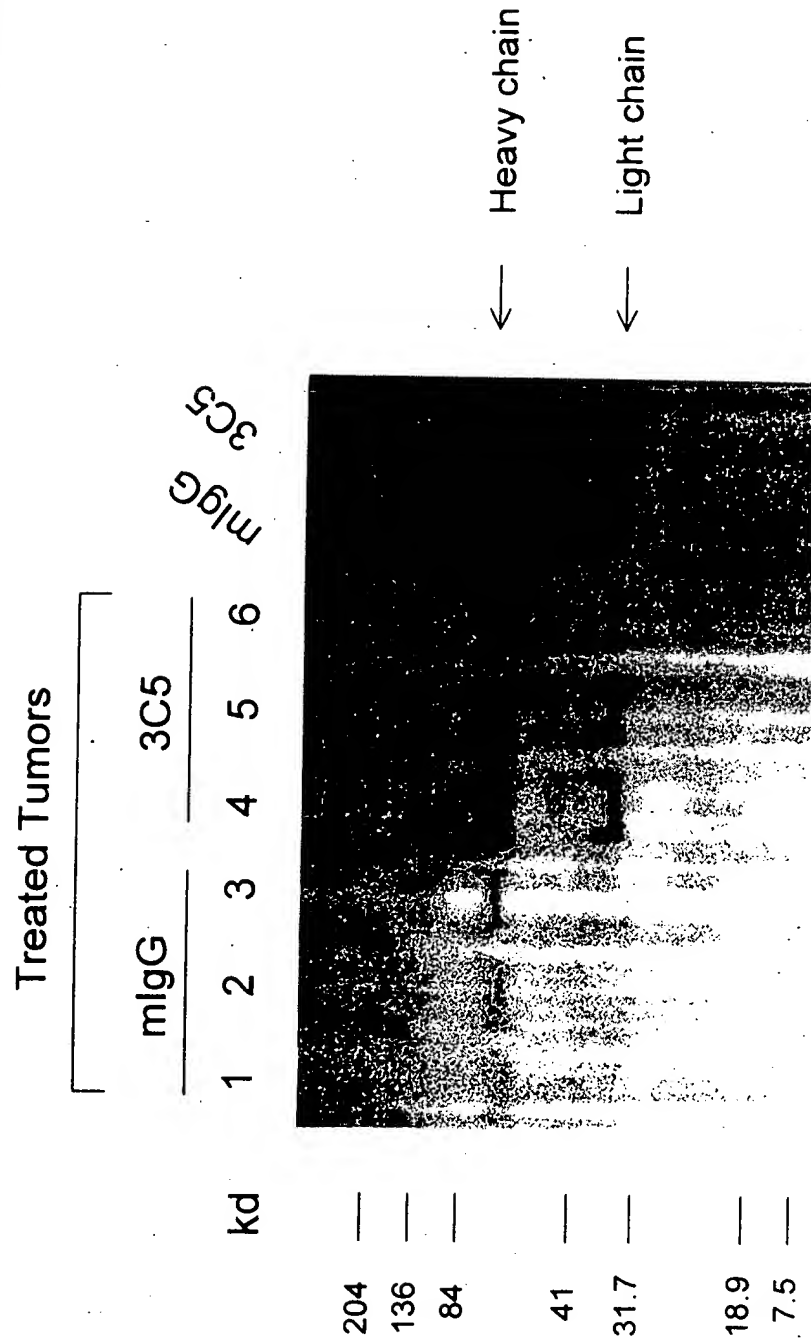
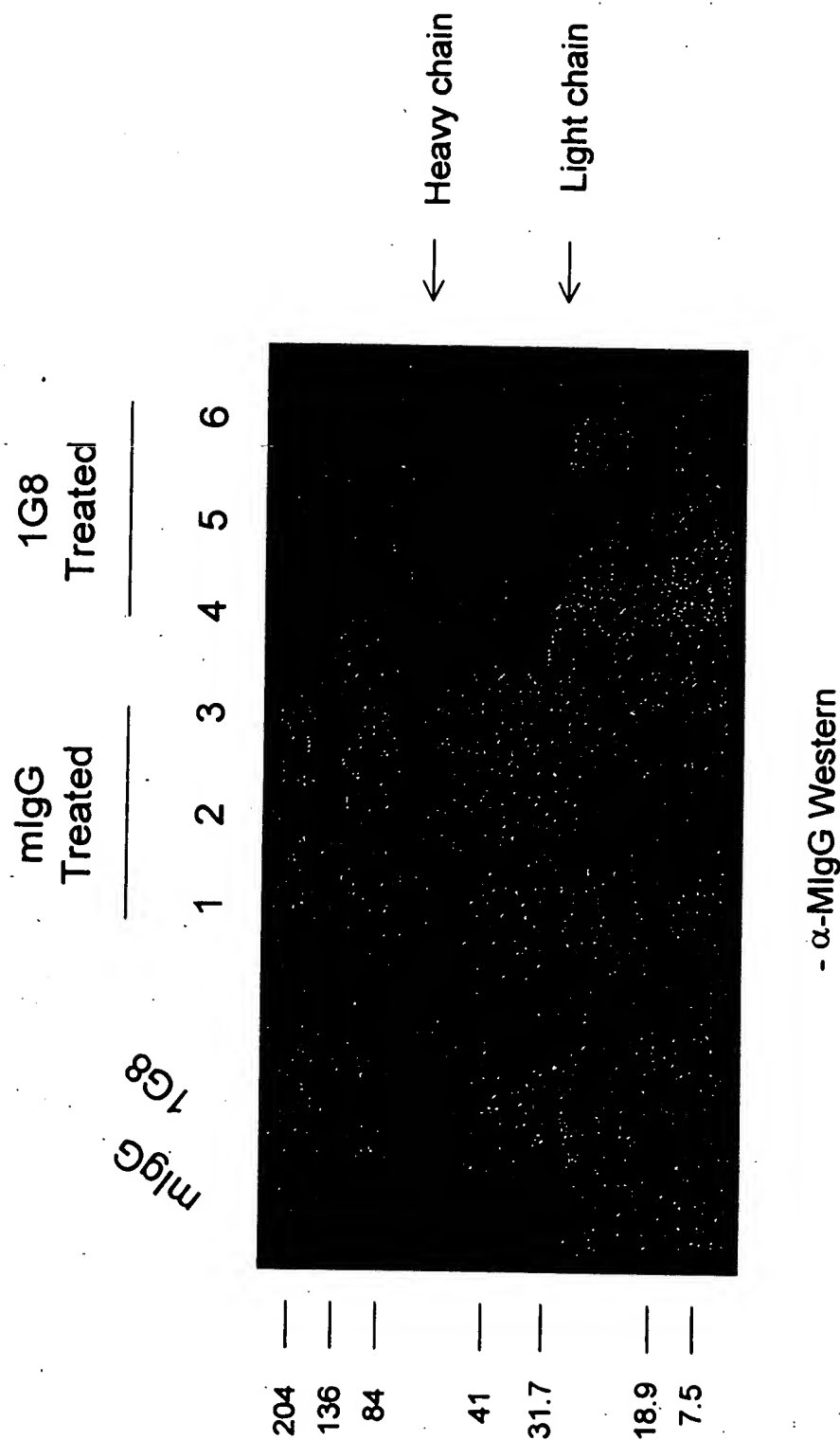


Figure 72

SPECIFIC TARGETING OF THE 1G8 ANTI-PSCA MAb TO ESTABLISHED LAPC-9 TUMORS



M thod: Mice bearing established LAPC-9 tumors (>100 mm³) were injected with either mIgG or the anti-PSCA MAb 1G8. Tumors were harvested a week later and made into protein lysates for Western analysis.

Figure 73